



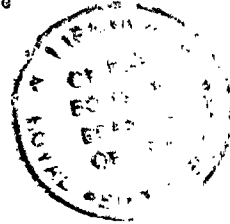
Report of the Haffkine Institute

For the Year 1929

BY

MAJOR L. A. P. ANDERSON, I.M.S.

Offg. Director, Haffkine Institute



[Price—Annas 4 or 5d.]

BOMBAY

PRINTED AT THE GOVERNMENT CENTRAL PRESS

1931

Obtainable from the Superintendent, Government Printing and Stationery,
Bombay, or through the High Commissioner for India, India House,
Aldwych, London, W.C.2, or through any recognized Bookseller

133226

BOMBAY BRANCH
OF THE
Royal Asiatic Society.

CONTENTS

	Page
Staff	1
Introduction	2
General Review	3
Haffkine's Plague Prophylactic Vaccine	18
Examination of Rats for Plague	20
Pharmacological Unit	21
Biochemical Unit	26
Chemical Department	28
Anti-rabic Department	30
Vaccine Department	32
Diagnostic Department	35
Pathological Section	36
Special Researches financed by the Indian Research Fund Association :-	
Anti-plague Inquiry	38
Inquiry on Maternal Morbidity and Mortality in Childbirth	49
Tuberculosis Inquiry	53
List of Papers published during the year	56
Appendix	58

REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1929

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Mr. S. N. Gore, L.M. & S. (Bom.)

Supernumerary Officer.

Major W. J. Webster, M.C., M.B., D.T.M. & H. (Eng.), I.M.S., Officiated as Assistant Director from 1st to 30th January and from 3rd August to 5th December 1929 ; Supernumerary Officer from 31st January to 2nd August and from 6th to 31st December 1929.

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Mr. R. G. Sathe, Veterinary Surgeon, from 11th September to 31st December 1929.

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 from 1st January to 31st March 1929.
 Mr. G. D. Chitre, L.M. & S., B.M.S. (Plague Research).
 Mr. N. P. Dalal, M.B., B.S., D.P.H., D.T.M. & H. (Eng.), B.M.S.
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 „ H. J. Parelkar—Engineer.
 „ Shaik Mahomedalli—Senior Media Maker.

Junior staff, subordinates and menials number 118.

INTRODUCTION.

In conformity with the policy regarding the compilation of anti-rabic statistics which was decided upon at a meeting of Directors of Pasteur Institutes held in Calcutta in December 1929, the figures from which these statistics are compiled have this year been taken from returns received six months after the completion of treatment, instead of three months as heretofore. This has necessitated delaying the publication

of this Report beyond the usual date. The period covered by the Report is, however, the same as in former years, namely, January to December.

During the year satisfactory progress has been made in the work of the Laboratory, and in addition to the very large amount of routine work dealt with in the vaccine and diagnostic departments, a great deal of very valuable research has been carried out which has fully maintained the high standard of former years.

It has been the custom for many years to commence this report with a general review of the work of the Institute during the year, while the more technical aspects are discussed in some detail in the body of the Report. This practice has been adhered to. With the wide distribution of the Report, among scientific and lay readers alike, it is desirable to include a brief account of the activities of the Institute generally, in less technical language, particularly for the benefit of those readers who though not medical men are interested in its work, while in the main Report the work of the different sections is presented in sufficient detail to appeal to those scientific readers who are more directly concerned with the various problems under review.

GENERAL REVIEW.

Plague Work.—An account of this side of the Institute's activities necessarily occupies a considerable part of the Annual Report. Under this heading are included plague vaccine manufacture and research thereon and a variety of research problems dealing with certain aspects of plague. Many of these investigations are being carried out at the instance of the International Plague Committee of the League of Nations and are aided by grants from the Indian Research Fund Association whose substantial help is gratefully acknowledged.

1. *Plague Vaccine.*—The demand for Haffkine's Prophylactic during the year under review, though showing a slight decrease as compared to the previous year, has been well up to the average, some million and a half doses having been despatched. It may be taken that the annual fluctuations in the issues of this vaccine fairly well represent fluctuations in the incidence of, though perhaps not the mortality from, plague throughout India and Burma, for there is every indication that the people are now fully alive to the value of this protective measure and do not hesitate to undergo inoculation when plague exists in or threatens their district.

The figures for the issue of plague vaccine to the different Provinces and Indian States in 1929 are somewhat unusual in that for the first time since 1923 the Bombay Presidency has been the largest consumer, with Mysore State second and the Punjab third. While it is probable that this is partly due to the activity of the Presidency health authorities, a large contributing factor was the somewhat heavy incidence of the disease in the Presidency which occurred during the months of July, August and September. This unusually early incidence caused for a time some embarrassment to the manufacturing department, but owing

to the very complete organization for vaccine production and aided by the new procedure immediately discussed in this Report, the difficulties were soon overcome and it is believed that no undue delay occurred in complying with all indents in full.

As forecasted in last year's Report, a Committee of plague research workers of the Institute was convened in February of the year under review, under the chairmanship of the Director, Lt.-Colonel (now Bt.-Colonel) F. P. Mackie, I.M.S., to consider certain problems in connection with the manufacture of plague vaccine, which had arisen as a result of the researches carried on during the past few years. This expert Committee was constituted as follows:—

- (1) Lt.-Colonel F. P. Mackie, I.M.S. (Chairman).
- (2) Major S. S. Sokhey, I.M.S.
- (3) Major W. J. Webster, I.M.S.
- (4) Dr. S. N. Gore.
- (5) Dr. B. P. B. Naidu.
- (6) Dr. C. R. Avari, B.M.S.
- (7) Dr. H. M. La Frenais, I.M.D.
- (8) Dr. N. P. Dalal, B.M.S.

For some years past the Directors of Public Health of the various provinces in India had been pressing for a solution of certain outstanding difficulties which they had experienced in their administration of the methods of plague prevention, and some of these criticisms were brought to a head at a meeting of the International Plague Committee which was called into being under the auspices of the League of Nations. This Committee met for the first time at Calcutta in December 1927 at the time of the Conference of the Far Eastern Association of Tropical Medicine. The Committee was presided over by Colonel Mackie, with Colonel (now Major-General) Forster, Director of Public Health of the Punjab, as the other Indian representative. The remainder of the Committee was composed of representatives from the various countries in the East and Far East.

Some of the questions involved had arisen more recently in correspondence with the Director of Public Health, United Provinces, who like other Directors of Public Health, had experienced great difficulty in forecasting in advance the amount of vaccine likely to be required, owing to the long period (3 months) required for the manufacture of Haffkine's vaccine. The necessity of ordering at least three months in advance large stocks of vaccine frequently resulted in a substantial loss to Government through wastage of vaccine from time expiry when the epidemic did not come up to expectations.

The problems involved were the following:—

- (1) Length of time during which Haffkine's Prophylactic retains its potency.
- (2) Whether the process of manufacture of the vaccine could be shortened down and with it the minimum notice required in order to meet unforeseen demands by any local Government.

(3) Whether agar vaccines could be used in place of Haffkine's broth vaccine as at present manufactured.

(4) Whether it was possible to reduce the toxicity of the vaccine and the reaction resulting from inoculation.

(5) Whether the dose of Haffkine's vaccine could be reduced.

The various research workers in this Institute had been for several years past at work on these and kindred problems and as a result of the Committee's discussions the following conclusions were reached in regard to the questions raised :—

(1) Haffkine's vaccine may continue to be used at the usual dose up to 18 months from the date of manufacture, but it may be considered to be effective up to a total period of two years provided that during that period the vaccine was kept in cold storage. This will not necessitate the vaccine being kept in cold storage during transit from one place to another.

(2) The process of manufacture can be cut down from twelve weeks to six weeks without marked loss of efficiency. It is proposed to maintain a stock in the Haffkine Institute of approximately three lakhs of doses and to inform indenting officers that not less than six weeks' notice should be given in advance, when they require a large bulk of vaccine.

(3) In view of the large bulk of evidence which has been accumulated on this point, from the experimental aspect, the Committee concluded that the substitution of agar vaccines for the present broth vaccine is not practicable. The results which have been obtained here as well as in other parts of the world where agar vaccines have been used, show that the immunizing value of such vaccine is markedly less than that of Haffkine's Prophylactic as manufactured here.

(4) There is no evidence to show that the toxicity of the vaccine at present prepared can be reduced, except that diminution which is observed to follow as a result of storage.

(5) Evidence has been brought forward by those who use Haffkine's vaccine in other countries that a satisfactory amount of immunity can be obtained from approximately half the dose which is used in India. The Committee, however, was of opinion that in view of the modifications in the manufacture of the vaccine which they have recommended, some of which may possibly result in a slight diminution of immunizing power, reduction of the dose used at present is inadvisable.

(6) In view of these modifications which they have recommended in the process of manufacture, the Committee propose to draw up a short form which Directors of Public Health will be asked to complete and return to this Institute for statistical examination. The statistical evidence which has hitherto been supplied has not always been in such a form that comparative results could be obtained. The Committee

considered it particularly necessary at this stage that figures relating to the immunizing value of Haffkine's vaccine should be more accurately returned.

The conclusions reached by the Plague Committee having been submitted to and approved by the local Government, a further meeting of the Committee, with the Acting Director as Chairman, was convened at the Haffkine Institute in April 1929 and the following proposals, based on the original Committee's findings regarding the future manufacture, use and method of indenting for plague vaccine were submitted to Government:—

(1) That plague vaccine may be used at the usual dose up to a period of two years from the date of manufacture, provided that during this period the vaccine has been kept in cold storage. This will not, however, necessitate the vaccine being kept in cold storage during transit from one place to another.

(2) That while the process of manufacture of Haffkine's vaccine will remain unchanged in its general method, the time required for the routine manufacture be reduced to six weeks instead of twelve weeks as heretofore. The greater part of this reduction is administrative. The actual period of incubation of the broth cultures, which is regarded as an essential factor in the preparation of Haffkine's Prophylactic, is only reduced by two weeks, i.e., from 6 to 4 weeks.

(3) That the dose of vaccine, until further information is available, remain unchanged, namely 4 cc.

(4) That in view of the reduction which can be effected in the time required for the preparation of the vaccine, indenting officers be asked to give not less than six weeks' notice in advance, when they require a large bulk of vaccine, instead of three months as heretofore. The regulation that the Institute will not take back vaccine which is time-expired, to hold good.

A form of "Report on Outbreak of Plague," reproduced herewith was drawn up as recommended by the Committee at its first meeting.

Report on Outbreak of Plague.

(To be returned to the Director, Haffkine Institute, Parel, Bombay.)

Population according to last census—

Date of report—

Locality of outbreak—

Date of commencement of outbreak—

Date of last case of Plague during epidemic under report—

Date of commencing inoculation—

Total number of persons inoculated—

Date of terminating inoculation—

A. Cases of plague occurring *before* inoculation was commenced, and the deaths* among these cases:—

Number of attacks—

Number of deaths—

B. Cases of plague occurring *after* inoculation was commenced, and the deaths* among these cases:—

Inoculated.

Uninoculated.

Number of attacks—

Number of attacks—

Number of deaths—

Number of deaths—

C. Details of deaths recorded among the inoculated* :—

[illegible]

*Notes.—(1) Under A only deaths occurring among those cases who were attacked before inoculation was begun should be shown here, irrespective of the date of death.

(2) Under B only deaths occurring among those cases who were attacked *after* inoculation was begun should be shown here.

(3) Under C if there is not sufficient space, further cases may be tabulated on a separate sheet of paper.

The above proposals were approved by the local Government and sanctioned by the Government of India who circulated the correspondence to all other Governments and Administrations for their information.

The procedure outlined above is now followed as a routine measure. Copies of the "Report on Outbreak of Plague" have been printed and are being supplied on demand to the Directors of Public Health of the different Provinces.

A number of these forms have already been returned completed, and while it is as yet too early to attempt any statistical analysis of the figures, the returns so far received indicate that valuable information is likely to be obtained from them in future.

2. *Researches in connection with the manufacture of plague vaccine.*—In its essential features the process of manufacture of plague vaccine is the same as that originally devised by Haffkine over thirty years ago ; continual research has, however, resulted in the introduction as a routine measure of control tests which enable the purity and sterility of this product to be guaranteed to a degree formerly not possible. The possibility of further advance is, however, not lost sight of and there has been no relaxation of research with a view to additional improvements.

Two directions in which further refinement appeared desirable are, first, the avoidance and detection of contaminations during the early stages of manufacture, so as to obviate the wastage of labour and

material that must necessarily occur when vaccine in the more advanced stages of preparation has to be discarded. Secondly, standardization of the finished product so that no vaccine is issued which does not conform to a certain standard of immunizing value.

It is on these lines that research in this department has chiefly been carried out during the year under review.

With regard to the first of these desiderata, the demand for stringent controls arises from the following fact. In the preparation of this vaccine stress has always been laid on the importance of employing a highly virulent strain of *B. pestis* and it has been for years the practice here to maintain the virulence of the strains (originally obtained by haemoculture from a human case of plague) by means of passage through susceptible rats. The "seed" for the preparation of the vaccine is, in fact, the heart blood of such a passage rat. Owing to the extreme ease with which contaminating organisms creep into such material constant vigilance is necessary to ensure the purity of the seed so that any contaminated samples may be discarded in the early stages of the manufacturing process. Dr. Gore has therefore continued his researches on the cultural reactions of *B. pestis* (which have been referred to in former issues of this Report) and has further elaborated his cultural controls in fluid and solid media so that *early* detection of contaminants, either in the "seed" or in the subsequent cultures used for the preparation of the vaccine is practically assured.

Cultures from the heart blood of a passage rat dead of plague ("Culture of the 1st remove") show by this method, in the great majority of cases, either *B. pestis* in pure culture or, even if contaminated, a growth of discrete colonies in which *B. pestis* predominates. It has seemed possible that if these colonies of the specific organism can be shown to retain full virulence in further pure subculture the difficulty of ensuring a pure "seed" would be greatly reduced. Numerous examinations of such cultures have demonstrated that the "culture of the 2nd remove", that is, discrete colonies obtained from a single colony taken from the "1st remove" culture, apparently exhibits no loss of virulence for rats. The liability of such a culture to contamination is immensely reduced and in practice it is only in such rare cases as when the contaminating organism is another member of the same (*Pasteurella*) group of organisms, whose cultural appearances on solid media closely resemble those of *B. pestis*, that difficulty seems likely to arise. These contaminants, however, so far as they have been met with up to the present, are eliminated either by the time of their appearance on solid media, by their haemolytic action, or by their cultural reactions in fluid media. It seems likely that this may subsequently prove to be the method of choice for obtaining the pure "seed".

As an ancillary research a study of the cultural reactions and biological processes of the *Pasteurella* group of organisms as a whole is being carried out by Drs. Gore and Naidu.

With regard to the second line of research it has long been considered desirable that vaccine issued by this laboratory should not only be of

guaranteed purity and sterility but should be of a standard immunizing value below which no vaccine would be regarded as fit for issue.

The standardization of vaccine as prepared by Haffkine's method presents considerable technical difficulties; unlike agar vaccines, this broth vaccine is not susceptible to any method of standardization by which the number of organisms contained in a given quantity of it can be determined, and the only form of standardization at present available is a purely empirical one dependent chiefly on the period of incubation of the broth cultures.

While this method probably gives successive brews of vaccine not differing markedly from one another in immunizing value, a more accurate estimation is certainly desirable.

It would appear that a biological method of standardization is the only one applicable to this vaccine and experiments have been in progress at this Institute for some years with a view to elaborating such a method, without, however, resulting in a test which is both dependable and capable of practical application as a routine procedure. This work has been continued, during the year under review, by Major Sokhey and Dr. La Frenais.

It was shown in last year's Report that the test virus (0.003 mg. plague spleen) hitherto in use for this work was inconstant as regards its content of plague bacilli and that tests carried out to estimate the immunizing value of duplicate samples of vaccine gave widely varying results. Major Sokhey and Dr. La Frenais, acting on the assumption, reasonable on *a priori* grounds, that given a strain of *B. pestis* of standard virulence the factor which determines the lethal or non-lethal effect of this strain in experimental animals is the number of organisms injected, attempted to devise a simple cultural technique by which a virus of known virulence and containing a known number of organisms could be prepared. Their preliminary work, reported last year, indicated the possibility of obtaining on these lines a virus whose minimal lethal dose (M.L.D.) could be determined within comparatively narrow limits and which could be used in multiples of the M.L.D. as a test virus for estimating the immunizing value of any particular sample of vaccine. These earlier tests indicated that the numerical factor was of great importance but by the close of the year a suitable dose of virus had not been determined.

In the development of their work the technique of standardizing the virus has been further elaborated and from the results of their experiments up-to-date it would appear that it is possible by comparatively simple cultural methods to prepare a virus of standard virulence whose bacterial content varies only within narrow limits and which when employed in suitable standard doses to determine the immunizing value of a vaccine gives closely corresponding figures with duplicated samples.

The accuracy of their method of estimating the bacterial content of the virus has been verified by checking the figures obtained with their colony count technique against direct counting under the microscope.

The figures for a large number of colony counts have proved consistent among themselves and showed a close correspondence—allowing for the differences in the two methods—when compared with direct bacterial counts.

Other researches carried out by Major Sokhey and Dr. La Frenais have been directed towards the determination of the ratio of growth of *B. pestis* on plain agar to that on blood agar, the effect on the growth and virulence of *B. pestis* of the addition of blood and blood products to the media used and the determination of the virulence of different strains of *B. pestis*.

It was shown recently by Gore that the addition of fresh rabbit's blood to media used for the cultivation of *B. pestis* markedly augmented the growth of this organism; co-incident with this augmentation of growth, however, there was, at any rate in fluid media, rapid loss of virulence. This fact has been confirmed. This loss of virulence, however, did not occur when blood "inactivated" by heat was added instead of fresh blood, while the growth was equally profuse. The addition of a haemoglobin preparation to the medium was also found to be as effective as fresh blood for enrichment purposes.

The addition of fresh blood to a medium precludes the possibility of subsequent sterilization, and the preparation of such media in a strictly sterile condition presents considerable technical difficulties in Bombay. These experiments, if confirmed and capable of general application, should go far towards solving one of the difficulties we have in preparing large quantities of enriched media for the cultivation of *B. pestis* and other haemophilic organisms if it should prove that subsequent sterilization of these media is possible without detriment to them. Experiments to determine this point are in progress.

3. *The Parasitology of Plague*.—Major Webster and Dr. Chitre have continued throughout the year their work on the flea factor in plague epidemiology. By observation of experimental epizootics among rats in the presence of pure bred fleas, they have been able to show that the climatic conditions met with in Bombay allow uninterrupted transmission throughout the year by both *Xenopsylla cheopis* and *X. astia*. They emphasise, however, that this experiment is purely qualitative and ignores certain important factors bearing on the relative importance of these fleas as transmitters in nature. The "blocked flea" phenomenon of Bacot has been investigated in its relation to tropical conditions and some interesting results have been obtained. It has been noted, *inter alia*, that the proportion of "blocked" fleas under the conditions of the experiment was extremely small, and that complete blocking is not an essential condition for infection.

The successful transmission for the first time in India by *X. brasiliensis* was referred to in last year's Report. In the continuation of these carefully controlled comparative transmission experiments, this species has proved a particularly regular transmitter of the disease. The importance of this species in the transmission of human plague has yet to be determined. It may be said here that so far as Major Webster

and Dr. Chitre could ascertain there is no difference in the attractiveness of man for the three species of *Xenopsylla*, all of which showed considerable longevity on an exclusive diet of human blood.

The proportion of infected fleas which continue to harbour the plague bacillus and the virulence of the organisms recoverable from these fleas has also been the subject of investigation. Dr. Gore has collaborated in this part of the inquiry.

4. *The Treatment of Plague.*—Two lines of investigation are being pursued in the endeavour to meet the demand for an effective therapeutic measure for the treatment of human plague. Both these researches were referred to in last year's Report and the work has been continued throughout the year under review.

(a) *The Preparation of Plague Serum.*

The first of these researches is the attempt of Dr. Naidu and his assistants to produce a potent anti-plague serum. It is true that more than one curative serum is already on the market, but unfortunately the results of treatment by these products have been uniformly disappointing in India.

Of the various factors that may be concerned in determining the potency of a curative serum it seemed likely that the following are of importance (i) the virulence of the organism used, (ii) the species of animal from which the serum is prepared, and (iii) the technique of preparation.

Dr. Naidu, taking the view that immunization of animals naturally susceptible to infection with organisms of the *Pasteurella* group—of which *B. pestis* is a member—with highly virulent local strains of the organism would probably result in the production of a more potent anti-serum than can be obtained from refractory animals, obtained last year from rabbits an anti-serum which gave most encouraging results when tested on animals experimentally infected with plague.

The ultimate aim of this work is, of course, the production of serum in sufficient quantity to be of practical use in the treatment of human plague; it was therefore necessary, as the next step, to determine whether by the same technique and with the same precautions as regards the species of animal used, a potent curative serum could be prepared from larger animals. For this purpose sheep and calves, both of which are susceptible to *Pasteurella* infections, were selected. Towards the end of the year immunization of some of these animals was complete and the curative value of the sera has been estimated. So far as experiments on animals are concerned, these sera appear to show a distinct advance on any sera hitherto introduced. Work is now in progress to prepare a large stock of serum with which it is hoped shortly to apply the crucial test, namely, its value in the treatment of human plague.

(b) *The Chemotherapy of Plague.*

Father Caius and Dr. Wadia are tackling the problem of the treatment of plague from another side, namely, from the chemotherapeutic aspect, and the work they began last year has been further developed. The drugs they have selected for this work are the halogenomercuriphenols,

all the twelve of which have been synthesized. Some of these compounds have been found to possess a very high germicidal power against *B. pestis in vitro*.

Work is now in progress to determine the safe dose of these drugs for experimental animals and to evaluate their therapeutic action in animals experimentally infected with plague.

THE PHARMACOLOGICAL DEPARTMENT.

Father Caius and Dr. Mhaskar have continued throughout the year their laborious studies of the indigenous medicinal plants. The study of *Gymnema sylvestre*, begun three years ago, has been completed and in addition 15 species of the genus *Terminalia* have been investigated. Some details of this work are given later in this Report.

An inquiry of this nature is extremely time-consuming and is moreover rendered more difficult owing to the confusion which exists in the botanical classification of the many plants of reputed medicinal value. Those who look for some startling discovery which is going to revolutionize medicine are doomed to disappointment. It may be said that so far few indigenous drugs of reputed value have been found on investigation to justify the claims made for them. The work is valuable, however, in that it should help to clear away the fog of mixed superstition and tradition on which the indigenous systems of medicine so largely depend, while at the same time making it possible to introduce to the medical profession on a true scientific basis such drugs of indigenous origin as may prove to be of real value.

Dr. Yodh, of the J. J. Hospital, continued to lend his valuable assistance to the clinical side of this inquiry.

THE BIOCHEMICAL DEPARTMENT.

The study of the anaemias in this country is considerably handicapped by the lack of normal standards for Indians in respect of certain constituents of the blood whose analysis is of importance in this condition. Major Sokhey and his assistants therefore devoted a good deal of time to the examination of normal healthy Indian men and women and have now established standards for certain of these factors. The standards will be of considerable value in future blood work.

The Anaemia Inquiry has been continued, but with some difficulty; the general use of liver treatment for the three severe types of anaemia met with in Bombay has brought about a very marked reduction in the number of fully developed cases formerly to be found in the hospitals, so that suitable material can now rarely be obtained.

From the great therapeutic value of liver treatment in pernicious anaemia, sprue and the severe anaemia of pregnancy, all of which respond equally well to this treatment, Major Sokhey considers that deficiency of some substance normally present in the liver of healthy animals is probably a common factor in the causation of the three diseases.

The basal metabolic rate of normal Indians has been further studied, with results confirming those reported last year.

The diagnostic work of this department has been considerably utilized and the value of biochemical analysis as an aid in the diagnosis of metabolic diseases is becoming more and more recognized by medical practitioners.

THE ANTI-RABIC DEPARTMENT.

The statistics relating to anti-rabic treatment this year, are based for the first time on health returns received six months after the completion of the treatment instead of three months as heretofore. This is in conformity with a resolution passed at a meeting of Directors of Pasteur Institutes held at Calcutta in December 1929 when the whole question of the compilation of anti-rabic statistics was fully reviewed.

The Paris strain of fixed virus is now being used exclusively for the treatment of cases at the Haffkine Institute. By the end of the present year (1930) we shall be in a position to judge whether the use of this strain has any advantage over the strains hitherto employed, and the results will be awaited with interest.

The number of patients treated, both at headquarters and at the different centres, differs little from last year, while the total mortality rate 0.73 per cent. for persons treated at headquarters, compares favourably with that of previous years.

Returns from the majority of the out-centres continue to be most unsatisfactory. Certain of the centres, and among them some of the largest and most important, have not obtained a single health return from their patients. It is obvious, therefore, that no statistics of any value can be obtained from all the mass of material available at these centres. This is a very deplorable state of affairs, and the question seriously arises whether it will not be desirable to close down certain of the defaulting centres altogether.

Part of the difficulty is undoubtedly due to the fact that until recently no grant existed for the purchase of stamped post cards for the purpose of these returns, but this difficulty has now been rectified. Again, certain centres complain that their personal is insufficient, and that the officer in charge of anti-rabic treatment has so much other work that he is unable to attend to the collection of returns. However this may be, the conclusion is unavoidable that a good deal of the trouble is due to lack of keenness and realization of the importance of securing adequate returns, since several large centres have succeeded in obtaining health reports from a very high percentage of their treated cases.

If the decentralization of Pasteur treatment is to be a real success, it is essential that the treatment should be carried out by a man trained in and employed mainly for this work. He should not be subject to transfer without very good reasons.

INQUIRY ON MATERNAL MORBIDITY AND MORTALITY IN CHILDBIRTH.

This inquiry has continued, somewhat erratically so far as Bombay is concerned, throughout the year under review. Although the

headquarters have remained at this Institute it was found necessary for various reasons to transfer a section of the work elsewhere for a considerable part of the year.

The work has been carried out under the general direction of Dr. (Miss) M. I. Balfour, except during August, September and part of October when she was on leave and her place was taken by Dr. (Miss) Wills.

Three main lines of inquiry have been followed. (a) The Anaemia of Pregnancy, (b) Osteo-malacia, and (c) The causes of early infant mortality with special reference to premature birth.

The etiology of the so-called Anaemia of Pregnancy has, as was the case last year, constituted the bulk of this research. A dietary survey among women of the different classes in Bombay has been completed by Dr. (Mrs.) Talpade together with a study of the different dietaries and the blood conditions associated therewith by Drs. Miss Wills and Miss Mehta. Wide differences in the dietaries of the different classes were found, and analysis of these indicated that an ill-balanced dietary, probably associated with vitamin deficiency, was a factor to be considered in investigating the cause of this malady.

While this dietetic study was proceeding, information was received from Colonel McCarrison, C.I.E., I.M.S., Director of Nutritional Research in Coonoor, that a severe anaemia had been observed by him in certain of his experimental animals fed on deficient diets. Dr. Wills, and subsequently Dr. Mehta, thereupon proceeded to Coonoor to investigate this matter and a number of animal experiments were carried out at the Nutritional Research Laboratories where Colonel McCarrison kindly placed all his resources at the disposal of the Enquiry.

The interesting results of this part of the research are given in some detail later on in this Report. Here it need only briefly be said that the onset of a severe anaemia could be determined in rats solely by the administration of deficient diets based on those commonly used by that class of women in Bombay in whom cases of anaemia are prevalent. Further investigation, however, showed that this experimental anaemia was associated with the presence in the blood of the Bartonella organism, with which all the stock rats were found to be infected. Bartonella anaemia has hitherto only been demonstrated in rats from which the spleen had been removed. It is believed that this is the first time this condition has been produced in the intact animal.

Subsequent to these experiments, many examinations of the blood of human anaemia cases failed to demonstrate the presence of Bartonella. It was found, moreover, that liver treatment which is so effective in human cases had no protective effect against Bartonella anaemia in splenectomized rats; it seems doubtful, therefore if the dietetic anaemia in rats, associated with Bartonella infection, has any direct bearing on the etiology of anaemia of pregnancy.

Further experiments are in progress in an attempt to produce anaemia by means of deficient diets, in animals not liable to Bartonella infection. •

Under (b) the blood chemistry of osteo-malacia patients has been investigated, both before and after treatment. The results will shortly be published.

The third line of research has consisted in a clinical enquiry by Drs. Balfour and Talpade. The question of syphilitic infection and the blood condition in relation to premature birth has been investigated.

The antenatal conditions of the mothers and the final result to the infants was studied in cases of poor weight infants with special reference to premature birth.

STILLBIRTH INQUIRY.

This investigation was started this year, in co-operation with the Maternal Mortality Inquiry, by Drs. Miss Thomson and Miss Patil. It was assisted by grants from the Lady Irwin Fund and, as part of the Maternal Mortality Inquiry, from the Indian Research Fund Association.

Although this Inquiry was designed to be completed in Bombay and was based on the Haffkine Institute where all facilities were given, it was found necessary to transfer the headquarters elsewhere for a good part of the year.

A most important branch of this work consisted in *post mortem* examinations of foetuses and stillborn infants, checked by hospital records of the causes of death, and for this it was essential that sufficient material should be made available by the co-operation of the local maternity hospitals. After some months' work in Bombay it became apparent that enough material to complete the investigation in reasonable time would not be forthcoming; the venue of the Inquiry was therefore changed to Madras. The headquarters were located at the King Institute of Preventive Medicine, Guindy, where all facilities were kindly given by the Director, and through the wholehearted co-operation of the Superintendents and staffs of the various maternity hospitals it was found possible to bring this part of the work to a conclusion.

Throughout the Inquiry much material was also received from hospitals in Calcutta.

The investigation has now been concluded and a full report is under preparation. An abstract of the work is given later in this Report.

TUBERCULOSIS INQUIRY.

This Inquiry which is being carried out by Dr. Soparkar under the auspices of the Indian Research Fund Association continued its work at this Institute throughout the year under review.

The question of the type of organism responsible for human surgical tuberculosis in India was further investigated, with results that confirmed last year's findings. So far as his experiments go, Dr. Soparkar concludes that in this part of India, at any rate, the bovine type of bacillus plays a negligible part in the causation of this form of

tuberculosis. The fact that tuberculosis of cattle in this Presidency is relatively infrequent would appear to be in conformity with this finding.

That this apparent freedom from risk of infection by the bovine type of bacillus may not hold good for other parts of India is, however, indicated by the fact that an investigation into the prevalence of cattle tuberculosis in the Punjab revealed a state of affairs closely approximating to that found in European countries, where a high percentage of cattle show definite evidence of tuberculous infection. It is evident, therefore, that here the risk of infection must definitely be considered, and an investigation into this question will shortly be undertaken.

Another important part of this Inquiry has been the study of B.C.G. (*Bacillus Calmette Guérin*) as an immunizing agent against subsequent tuberculous infection. This prophylactic measure, which is now being employed in Europe for the protection of children, has given rise to much controversy. The details of the work carried out on this problem by Dr. Soparkar and his assistants are given in the body of this Report. Their experiments on rabbits indicate that in susceptible animals B.C.G. may exceptionally prove virulent and produce fatal tuberculosis, while in their hands prophylactic inoculation of B.C.G. afforded meagre protection, evidenced only by prolongation of life in vaccinated animals subsequently infected with the smallest dose of virulent organism.

THE INDIAN RESEARCH FUND ASSOCIATION.

The Indian Research Fund Association has again contributed generously to the research work carried out at this Institute. Three major Inquiries, the Plague Inquiry, the Tuberculosis Inquiry and the Inquiry on Maternal Morbidity and Mortality in Childbirth have been financed by grants from this fund, amounting in the aggregate to 1½ lakhs of rupees.

Bombay has every reason to be grateful to the Government of India from whom these grants ultimately derive, for without this assistance these researches, all of which are dealing with problems of considerable local importance, could not be carried on.

A new animal shed, giving badly needed accommodation for the larger animals employed in certain of the Inquiries, has also been built at the Institute with Association funds.

THE SOCIAL LIFE OF THE INSTITUTE.

The Critics Club.

This Club was founded in 1924 by the Director to promote social intercourse between the staffs of the different departments.

Meetings are held periodically, when papers are read and any researches carried out in the Institute are freely discussed and criticized. The special feature of these meetings is that they take the form of social, not formal, gatherings.

Papers on the following subjects were read and discussed during the year: (1) The importance of testing bacteria for the production of nitrite, by Gore; (2) A comparative study of the dried barks of the commoner Indian species of the genus *Terminalia Linn.*, by Mhaskar.

The Tennis Club which is a branch of the Critics Club has continued to flourish; the annual tennis tournament for the staff produced some keenly contested matches and provided a series of most enjoyable evenings.

The Cricket Club.

This club is now a well established feature of our social life. It has had a very successful season and the record of matches played bears witness to the keenness and improved play of our eleven. The credit of this is chiefly due to our enthusiastic cricket captain, and to the skill and interest he showed in coaching the players and leading the team. During the season 35 matches were played, of which 20 were won, 12 lost, and the remainder drawn. Financially the club just holds its own and for this we have to thank the generosity of the staff in subscribing to its upkeep.

The Badminton Club, started this year at the instance of the clerical staff, provides exercise and amusement for those who do not play tennis.

During the year we had the pleasure of entertaining the distinguished members of the Malaria Commission of the League of Nations, who toured India during the cold weather. In August, during their first visit to Bombay, a garden party was given in their honour and was attended by a large number of prominent members of the local medical profession. In December, when the Commission returned to Bombay at the conclusion of their tour, an informal conversazione was held at the Institute, followed by tea in the Director's garden. The members of the Commission were much interested in the work of the Institute and expressed their pleasure in all that they had seen.

In December 1929 Dr. Serge Voronoff, the well known exponent of testicular grafting, visited the Institute and gave a lecture on his work, illustrated by lantern slides and cinema films. Invitations to the lecture were sent to all the medical institutions in Bombay and about 100 medical men attended.

During the prevalence of plague in this Presidency in October 1929, the services of Mr. J. Mallett, Technical Assistant of this Institute, were placed at the disposal of the Kolhapur Durbar for ratting operations in connection with the plague preventive measures carried out in anticipation of His Excellency the Viceroy's visit to that State. A letter of appreciation of his services was received from the Durbar.

Casualties.—In January 1929 we had to deplore the untimely death of Sub-Assistant Surgeon Jemadar Shamsheer Jung, I.M.D. This officer, in the course of his work on the Plague Inquiry, had the misfortune to contract the disease, the attack rapidly proving fatal. By his death a most promising officer has been lost to the Service.

On 1st April of the year under review Khan Bahadur Dr. C. R. Avari, B.M.S., retired after 35 years' continuous service, of which 24 years

were spent at this Institute. The acting Director would place on record his appreciation of the loyal and valuable services Dr. Avari has rendered during his long association with this Institute.

In concluding this review of the work of the Institute the writer wishes to express his thanks to his colleagues and to the whole staff for their unfailing loyalty and co-operation through which his work has been rendered both pleasant and profitable.

HAFFKINE'S PLAGUE PROPHYLACTIC VACCINE.

1. *Manufacture*.—The value of anti-plague inoculation is now universally recognized. The popularity of this measure is reflected in the output from this Laboratory, which has shown a steady increase since the prophylactic was first introduced.

From the opening of the Laboratory to the end of 1929, 32,617,829 doses have been sent out. The output of the vaccine has been as follows :—

Years					Doses
1897-1901	..	..	..	..	2,867,041
1902-1906	..	..	..	..	3,455,537
1907-1911	..	..	..	..	3,584,262
1912-1916	..	..	..	..	4,567,366
1917-1921	..	..	..	..	6,667,894
1922-1926	..	..	..	..	7,202,636
1927-1929	..	..	..	..	4,273,093

During the year 1929, the output was 1,487,374 doses ; the number of doses of anti-plague vaccine despatched monthly during the past two years are detailed below :—

Month					1928	1929
January	..	..	..	..	164,072	217,554
February	..	..	..	..	365,196	265,347
March	..	..	..	..	284,311	74,094
April	..	..	..	..	202,526	33,697
May	..	..	..	..	60,264	3,876
June	..	..	..	..	16,464	4,404
July	..	..	..	..	36,518	85,440
August	..	..	..	..	56,598	176,160
September	..	..	..	..	196,356	201,852
October	..	..	..	..	130,200	155,496
November	..	..	..	..	119,796	178,236
December	..	..	..	..	228,108	91,218
Total number of doses ..					1,960,409	1,487,374

2. *Distribution.*—The geographical distribution of the vaccine in India and to places outside India during the years 1928 and 1929 is shown below :—

				No. of Doses	
				1928	1929
<i>British India—</i>					
Bombay Presidency	..	..	..	255,787	347,920
Madras Presidency	..	..	..	130,416	51,792
Bengal, Bihar and Assam ..	..	..	..	36,738	43,050
United Provinces of Agra and Oudh	..	..	..	521,596	173,334
The Punjab	..	..	..	466,494	200,646
North-West Frontier Provinces	..	..	..	2,688	666
Central Provinces and Berar ..	..	..	..	106,110	57,750
Delhi Province	..	..	..	1,986	
Burma	..	..	..	80,514	65,316
Total ..				1,602,329	940,474
<i>Indian States—</i>					
Kathiawar	..	..	..	6,108	7,224
Southern Maratha Country ..	..	..	..	18,048	135,612
Savantwadi	..	..	..	1,770	204
Baroda	..	..	..	2,256	2,142
Cutch	..	..	..	7,860	
Hyderabad	..	..	..	120,750	100,818
Mysore	..	..	..	136,662	252,702
Travancore	..	..	..	18,402	9,024
Benares	..	..	..	2,400	1,266
Patiala	..	..	..	20,214	10,020
Jhind	..	..	..	3,012	
Faridkot	..	..	..	1,008	204
Jammu	..	..	..	2,658	1,500
Bhopal	..	..	..		102
Jaipur	..	..	..	462	204
Bikaner	..	..	..	402	
Jodhpur	..	..	..		156
Indore	..	..	..	102	204
Rewa	..	..	..	2,016	
Dhar	..	..	..	12	
Dewas Junior	..	..	..	12	
Darbhanga	..	..	..	504	150
Rampur	..	..	..	3,204	
Alwar	..	..	..	408	
Maihar	..	..	..	750	
Sandur	..	..	..	234	72
Ramandrug	..	..	..	810	
Tonk, Rajputana	..	..	..	54	
Nabha	..	..	..	204	
Butlam	..	..	..	72	
Gwalior	..	..	..		6
Total ..				350,394	521,610
<i>Portuguese Territory in India</i>				120	36
<i>Countries outside India—</i>					
Abyssinia-Addis Ababa	..	..	..	2,004	
British Somaliland Berbera ..	..	..	..	2,010	
<i>China—</i>					
Siensyu Fukien	..	..	..	504	498
Hinghwa Foochow	..	..	..	2,202	3,000
<i>Straits Settlements—</i>					
Singapore	..	..	..	846	
Penang	..	..	..		504
Egypt-Cairo	..	..	..		21,252
Total ..				7,566	25,254

				No. of Doses	
				1928	1929
British India	..	..	..	1,602,329	940,474
Indian States	..	..	..	350,394	521,610
Portuguese Territory in India	..	..	..	120	36
Countries outside India	..	..	..	7,566	25,254
Total number of doses				1,960,409	1,487,374

EXAMINATION OF RATS FOR PLAGUE.

1

Rats received at the Institute from the Municipality during 1929.

Month				Alive	Dead	Total
January	..	..	..	21,956	36,514	58,470
February	..	..	..	11,062	18,854	29,916
March	..	..	..	16,621	29,641	46,262
April	..	..	..	18,877	29,359	46,236
May	..	..	..	19,329	28,962	48,291
June	..	..	..	18,029	30,866	48,895
July	..	..	..	21,416	30,486	51,902
August	..	..	..	22,389	34,364	56,753
September	..	..	..	24,534	33,311	57,845
October	..	..	..	25,596	37,930	63,526
November	..	..	..	24,264	35,092	59,356
December	..	..	..	25,056	36,250	61,306
Total				247,129	381,629	628,758

2

Examination of dead rats for plague during 1929.

Month				Dead rats examined	Plague infected rats	<i>Rattus norvegicus</i>	<i>Rattus rattus</i>
January	..	..	..	27,960	54	47	7
February	..	..	..	17,162	22	21	1
March	..	..	..	17,363	67	57	10
April	..	..	..	19,963	85	83	2
May	..	..	..	20,947	67	62	5
June	..	..	..	18,860	33	30	3
July	..	..	..	22,859	25	25	0
August	..	..	..	24,520	28	24	4
September	..	..	..	22,541	27	26	1
October	..	..	..	27,083	32	29	3
November	..	..	..	24,160	42	41	1
December	..	..	..	24,739	30	26	4
Total				268,157	512	471	41

Relation of human plague deaths to plague deaths among rats.

Month	Human plague deaths	Rats examined <i>post mortem</i>	Plague infected rats	Percentage mortality
January	3	27,960	54	0·2
February	3	17,162	22	0·1
March	5	17,363	67	0·4
April	4	19,963	85	0·4
May	1	20,974	67	0·3
June	4	18,860	33	0·2
July	..	22,859	25	0·1
August	2	24,520	28	0·1
September	..	22,541	27	0·1
October	1	27,083	32	0·1
November	..	24,160	42	0·2
December	..	24,739	30	0·1
Total ..	23	268,157	512	0·19

PHARMACOLOGICAL LABORATORY.

**A. Indigenous Drugs.*

I. *Gymnema sylvestre*.—The study of this plant was completed in the course of the year, and the summary of the results is as follows :—.

(1) The air-dried leaves yield, after ignition, 11·45 per cent. of inorganic matter which was found to contain per cent. : “alkali” 46·76 ; phosphoric acid 6·73 ; ferric oxide 5·44 ; manganese 1·31.

(2) They do not contain any water-soluble or alcohol-soluble substance which destroys glucose *in vitro* ; nor do they yield any chemical body resembling insulin.

(3) They contain the two hydrocarbons hentriacontane and penta-triacontane, chlorophyll *a* and *b*, phytol, resins, tartaric acid, inositol, anthraquinone bodies, and “gymnemic acid”.

(4) “Gymnemic acid” is a highly complex mixture which may be fractionated by successive treatment with petroleum ether, ether, chloroform, ethyl acetate, and alcohol. It is evident that none of these fractions represents a homogeneous substance.

(5) The leaves of gymnemic acid have no bactericidal action *in vitro*. Their toxic action is manifested by loss of appetite, diarrhoea and asthenia, which, in animals is followed by paresis of the limbs, irregularly high temperature, and death mostly without convulsions due to respiratory failure.

(6) They stimulate the heart and circulatory system, increase urine secretion, and augment the contractions of the uterus.

(7) They cause hypoglycæmia which sets in soon after the administration of the drug whether by mouth or by injection, lasts for

a variable time, is not necessarily proportionate to the dose and is never excessive. The drug has no direct action on carbohydrate metabolism, and appears to act indirectly through stimulation of insulin secretion of the pancreas.

(8) The presence of anthraquinone derivatives accounts for the laxative action of the leaves.

(9) The drug appears to be useful in checking glycosuria when administered in doses of 2 to 4 grammes of dry leaf.

(10) The ease of administration, the stomachic, stimulant, diuretic, and laxative properties, together with the mineral content mark the drug as a prescription for the treatment of glycosuria.

(11) The root is useless as an antidote to snake venom.

II. *Terminalia*, Linn.—The bark of *T. Arjuna* is considered throughout the length and breadth of India as a very valuable cardiac tonic; but the results we obtained with “arjun” from the local bazaar showed (1926) that, except for diuretic properties, the drug was pharmacodynamically inert. We then procured authentic samples of both *T. Arjuna* and *T. tomentosa*, and found that the two barks were so very similar in appearance that confusion was not only possible but pardonable. The bark of *T. Arjuna* was found to be inert while that of *T. tomentosa* had an action on the heart (1926). Moreover the examination of a very unlike bark, that of *T. Chebula* (1927), revealed, together with diuretic effects, an action on the heart almost as marked as that of *T. tomentosa*. We accordingly decided upon a comparative study of the Indian species of the genus *Terminalia*.

We were not able to procure all the twenty Indian species and had to limit our study to fifteen.

(1) The dried barks exhibit a great variability of forms; nevertheless we find that the pink coloured barks of *Arjuna*, *coriacea*, *pallida*, *paniculata* and *tomentosa* are generally mistaken for one another and that they are being exhibited and sold indiscriminately as “arjun”. We have prepared a provisional key for the identification of the dry barks of the fifteen commoner species.

(2) Except for the presence of iron in *pyrifolia* and *travancorensis* the ashes have much the same composition and are remarkable for a high and fairly constant percentage of calcium and carbon dioxide.

(3) There is no apparent interdependence between the pharmacodynamic potency of the barks and (1) the percentage of potassium, and calcium, or (2) the nature of the tannins.

(4) The barks of *T. Manii* and *T. travancorensis* are pharmacodynamically inert.

(5) The pharmacodynamically active barks are either (i) mild diuretics—*T. Arjuna*, *T. belerica*, *T. pallida*—or (ii) fairly potent cardiac stimulants—*T. bialata*, *T. coriacea*, *T. pyrifolia*—or (iii) have both diuretic and cardiotonic properties—*T. Catappa*, *T. Chebula*, *T. citrina*, *T. myriocarpa*, *T. Oliveri*, *T. paniculata*, *T. tomentosa*.

III. At the 19th Session of the All India Ayurveda Mahasammelan, held at Nasik 18th-22nd May 1929, we noticed the following exhibits :— (a) *T. tomentosa* labelled “sanskrit”: arjun; “marathi”: pandhari madat; “canarese”: bile matti. (b) *T. coriacea* labelled “skt”: arjun bheda; “mar”: arjun bheda, ain. (c) *T. coriacea* named “skt”: rakta arjun; “mar”: tambada arjun; “can”: kempmatti. (d) *T. coriacea* named “skt”: arjun; “mar”: arjun. A bark exhibited as *Terminalia paniculata* (sic) was not that of a *Terminalia*.

Two other exhibits particularly attracted our attention. One of them, labelled “sanskrit”: arkapushpi; “marathi”: vakhandi, was a fine specimen of *Cryptolepis Buchanani* Roem and Schult with leaves, flowers, and fruits: yet, the Sanskrit name was that of *Gynandropsis pentaphylla* DC., and the Marathi name that commonly used for *Gymnema sylvestre* Br. The second specimen was labelled “sanskrit”: meshashrangi, one of the Sanskrit names for *G. sylvestre*, but was actually *Daemia extensa* R.Br.

Through the courtesy of the Managing Committee we were able to take away 327 exhibits which we carefully examined with the following results :—(a) 204 specimens were identified botanically: (i) of these 159 were named correctly in the sense that the latin and vernacular synonyms tallied—they were all common plants; (ii) in 18 cases one or the other of the vernacular names did not apply; (iii) in the remaining 27 cases the vernacular names were those given to other plants of the same or even of different orders. (b) Insufficiency of material or the bad condition of the specimen made the exact botanical identification of 123 samples impossible: (i) but enough information could be gathered to say that 47 were wrongly named in the sense that the vernacular names did not apply to the material in hand; (ii) in 38 cases the vernacular synonyms could not be found in the literature; (iii) in 15 cases the vernacular names were found, but the plants to which those names should be applied have not been traced; (iv) nothing could be made out of the remaining 23 specimens.

The findings may be summarised in the words of Mr. Deodhar Shastri, President of the Reception Committee, ... “even though we know the names of drugs, still in practice we cannot identify the correct specimen.”

IV. We submitted to the Government of Bombay a summary of the results we have obtained with Indian medicinal drugs, indigenous or imported, during the period beginning July 1917 and ending November 1929.

The results recorded mark two different stages in the progression of an inquiry into the therapeutic value of such drugs.

The first part of the investigation (July 1917-March 1923) was carried out in the Central Jail at Trichinopoly, under the auspices of the Indian Research Fund Association. It dealt with anthelmintics, for the controlled population of a jail is specially suited to the study of worm remedies. .

Shortly after the opening of the Pharmacological Laboratory in Bombay all hopes of continuing the work begun at Trichinopoly had to be abandoned owing to the Government Resolution that such experiments should not be made on prisoners (No. 8146, Home Department, Bombay Castle, 2nd July 1925).

As the conditions prevailing in the local hospitals were found suitable for the controlled treatment of dysenteries and diarrhoeas—and, to some extent, malaria,—full advantage was taken of the situation to determine the therapeutic value of drugs credited with antidiarrhoeal and antimalarial properties.

Attempts at controlling the treatment of diabetic troubles proved futile, and the possibility of treating cardiac disorders never occurred. In such circumstances the conclusions as to the therapeutic value of drugs in these two conditions had to be drawn exclusively from experiments on animals.

The following 63 plants have been examined:—

1. *Acalypha indica* Linn.; 2. *Aloe vera* Linn.; 3. *Alstonia scholaris* R.Br.; 4. *Apium graveolens* Linn.; 5. *Areca catechu* Linn.; 6. *Artemisia absinthium* Linn.; 7. *A. maritima* Linn.; 8. *Aspidium Filix-mas* Swartz; 9. *Blumea balsamifera* D. C.; 10. *B. densiflora* D. C.; 11. *Brayera anthelmintica* Kunth; 12. *Butea frondosa* Roxb.; 13. *Carum copticum* B. & H.; 14. *Caryophyllum aromaticum* Linn.; 15. *Cinnamomum* Cassia Blume; 16. *C. iners* Reinw.; 17. *C. Tamala* Nees; 18. *C. zeylanica* Breyn.; 19. *Cocos nucifera* Linn.; 20. *Croton Tiglium* Linn.; 21. *Embelia ribes* Burm.; 22. *E. robusta* Roxb.; 23. *Eucalyptus citriodora* Hook.; 24. *E. globulus* Labill.; 25. *Foeniculum vulgare* Gaertn.; 26. *Garcinia Mangostana* Linn.; 27. *Garultheria fragrantissima* Wall.; 28. *Gymnema sylvestre* Br.; 29. *Holarrhena antidysenterica* Wall.; 30. *Illicium verum* Hook. f.; 31. *Mallotus philippinensis* Muell-Arg.; 32. *Melaleuca leucadendron* Linn.; 33. *Melia azadirachta* Linn.; 34. *Mentha arvensis* Linn.; 35. *M. piperita* Linn.; 36. *Nyctanthes Arbor-tristis* Linn.; 37. *Petroselinum sativum* Hoffman; 38. *Picrasma quassioides* Benn.; 39. *Pimpinella Anisum* Linn.; 40. *Pinus longifolia* Roxb.; 41. *Piper Betle* Linn.; 42. *Punica granatum* Linn.; 43. *Ricinus communis* Linn.; 44. *Ruta graveolens* Linn.; 45. *Sassafras officinale* Nees; 46. *Terminalia Arjuna* W. & A.; 47. *T. belerica* Roxb.; 48. *T. bialata* Steud.; 49. *T. Catappa* Linn.; 50. *T. Chebula* Retz.; 51. *T. citrina* Fleming; 52. *T. coriacea* W. & A.; 53. *T. Manii* King; 54. *T. myriocarpa* Heurck. & Muell-Arg.; 55. *T. Oliveri* Brandis; 56. *T. pallida* Brandis; 57. *T. paniculata* Roth.; 58. *T. pyrifolia* Kurz.; 59. *T. tomentosa* W. & A.; 60. *T. travancorensis* W. & A.; 61. *Vernonia anthelmintica* Willd.; 62. *Wrightia tinctoria* R.Br.; 63. *W. tomentosa* Roem. & Schultes.

Of the numerous drugs used daily in India for the removal of worms none can be recommended as an anthelmintic. *Carum copticum* and *Artemisia maritima*, though themselves practically inert, are the source

of two most powerful anthelmintics : thymol against hookworms and santonine against roundworms, respectively. The discovery of hitherto unknown anthelmintic properties in oil of sassafras, oil of cloves, oil of anise, oil of wintergreen was the natural consequence of the knowledge acquired by the two workers of the correlation between therapeutic properties and chemical composition.

There is little to recommend the official *Alstonia scholaris* in the treatment of either malaria or diarrhoeas. On the other hand the bark of *Holarrhena antidysenterica* has an antidysenteric value which compares favourably with that of any of the remedies now in vogue. *Garcinia Mangostana* may also be safely recommended for its antidiarrhoeal properties.

The chemical composition and the pharmacodynamic properties of *Gymnema sylvestre* justify its use to check glycosuria.

The discovery of cardiotonic properties in a number of Indian species of the genus *Terminalia* is of more than academic interest. The pharmacodynamic activity of *T. citrina* and *T. Oliveri* as cardiac stimulants call for a thorough clinical study ; but, alas for the want of facilities !

B. Chemotherapy of Plague.

Vide infra under Anti-plague Inquiry.

C. Earth-eating and Salt-licking.

Nine samples of soils from different parts of India and Burma were analysed. Six were salt-licks, two common earth, and one earth for human consumption.

D. Scorpion Venom.

A sting from *Butheolus melanurus* Kessler resulted in a sharp burning pain which lasted for a few seconds and was followed by throbbing and dull pain. Within two minutes from the time of the injection all that was noticeable was an indefinite feeling of discomfort in the region of the sting ; this persisted for one hour and twenty minutes. The action of the venom was entirely local. The amount of venom is so very small as not to yield to any of the methods of extraction.

The undermentioned quantities of poison were collected from the live specimens :—

			Number of Scorpions.	Weight of dry venom in milligrams.
<i>Butheolus bicolor</i>	..	..	1	0·0
„ <i>melanurus</i>	..	..	5	0·0
<i>Buthus australis</i>	..	..	3	2·0
„ <i>pachyurus</i>	..	..	57	25·5
„ <i>rugiscutis</i>	..	..	12	2·6
„ <i>tamulus</i>	..	..	372	810·8

		Number of Scorpions.	Weight of dry venom in milligrams.
<i>Palamnaeus bengalensis</i>	..	2	9.7
„ <i>fulvipes</i>	..	55	191.0
„ <i>gravimanus</i>	..	4	14.6
„ <i>liurus</i>	..	20	13.6
„ <i>longimanus</i>	..	5	18.5
„ <i>phipsoni</i>	..	10	48.6
„ <i>swammerdami</i>	..	45	659.0
„ <i>xanthopus</i>	..	5	16.3
<i>Scorpiops asthenurus</i>	..	5	5.6
„ <i>montanus</i>	..	7	3.6
<i>Isometrus europaeus</i> (vesicles)	..	10	7.9

E. Chemical Analysis.

A sample of oil labelled “Vegetol Oil” was received from the N. M. Mental Hospital, Naupada, Thana. The oil was found to be free from cholesterol.

BIOCHEMICAL UNIT.

Haemoglobin, Color Index, Saturation and Volume Index Standards.—During the study of anæmias both in this Unit and in conjunction with the Inquiries on Sprue and Maternal Mortality, it was realised that no normal standards for Indians were available against which pathological findings could be compared. Even the European standards to be found in text books could not be pressed into use. The normal red cell count of 5 millions universally given in the text books, it transpires, was based on the result of four determinations only. More recent surveys carried out with instruments of greater precision have given different results. Osgood, for example, recently gave an average count of 5.4 millions based on the examination of 137 healthy young American males. When the question of hæmoglobin was considered, the situation was found to be still worse. The normal standard given by various authorities varies from 13.77 g. to 17.2 g. per 100 cc. blood (the cent. per cent. figure of various instruments is as follows :—Dare 13.77 g., Haldane 13.80 g., Oliver 15.00 g., Von Fleischl Miescher 15.80 g., Tallquist 15.80 g., Newcomer 16.92 g., and Sahli 17.20 g., per 100 cc. blood). When it is realised that the hæmoglobin figures are invariably expressed as so much per cent. of the normal, the resultant confusion can be easily imagined. Similarly Colour Index, Saturation Index, and Volume Index figures given in the literature are not comparable with each other owing to the different techniques used by the various authors.

During the year under review Major Sokhey and his assistants have examined 121 healthy young Indian men and 101 healthy young Indian women living in Bombay. Realising that the hæmoglobin determinations as at present carried out in clinical work give inaccurate results—some of the methods in use give an error as high as 20 per cent.—all their hæmoglobin determinations have been made with Van Slyke's Constant Volume Blood Gas Apparatus. This method gives a very high degree

of accuracy, but is time consuming and is not suited for clinical work; parallel determinations have therefore been carried out by an acid hematin colorimetric method. The cell counts were made with chambers with Levy-Neubauer rulings, tested by the Bureau of Standards, and specially standardized pipettes. Cell volumes were determined by centrifuging blood for 30 minutes at 4,500 revolutions per minute. The technique described by Osgood was used in order to get results comparable to his. The following averages have been obtained :—

	Men	Women
Red cell counts, millions per c.mm. . .	5·11	4·47
Haemoglobin—grams per 100 cc. . .	15·36	12·99
Volume of cells, cc. per 100 cc. . .	41·72	36·27

The results of this inquiry will be published shortly.

Anaemia Inquiry.—During the year it has not been possible to add materially to the number of cases of Pernicious Anaemia, Sprue and the Severe Anaemia of Pregnancy under investigation for the purposes of this Inquiry. The use of liver preparations has become so general that fully developed cases of these conditions have become rare—at least in the hospital wards.

As was indicated in the last year's report, the resemblances between these conditions are more striking than the differences, except perhaps as regards the presence or absence of free hydrochloric acid in the gastric contents. Severe oligocythaemia with a high colour index, anisocytosis with marked macrocytosis is common to the three conditions. Smear pictures, if not always exactly identical at the start, became so after the patients had received liver treatment for a short time.

The importance of the absence of free hydrochloric acid in the gastric contents in Pernicious Anaemia as a diagnostic feature distinguishing this disease from Sprue and the Severe Anaemia of Pregnancy is difficult to assess. The differences noted are quantitative but the nature of the change seems to be the same.

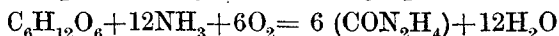
Nervous symptoms have not helped much; these symptoms were absent in a large proportion even of Pernicious Anaemia cases. On the other hand some other workers have reported cord changes in typical cases of sprue.

The equally good response of these three conditions to liver treatment irresistibly suggests the presumption that at least one and perhaps the most important etiological factor is common to all three diseases, and that this factor is a deficiency of some substance normally present in the liver of healthy animals and certain other living tissues. The deficiency may be due to the lack of this substance in the required amount in the diet of the patient, or it may result from the inability of the patient to assimilate the substance when it is present only in small amounts in the food, or finally it may be created by the extra demand associated with pregnancy. Whatever may be the immediate etiological cause or causes, the fact remains that when large amounts of the substance are added to the diet of the patient in the form of liver extract, the

deficiency is made good without the necessity of having to deal with these immediate causes. There is thus a strong indication that we are dealing with a deficiency disease; the actual clinical picture resulting from the deficiency apparently depends on some factor or factors individual to each patient.

Basal Metabolism.—During the year Benedict's "Field Respiration apparatus for a Medical and Physiological Survey of Racial Metabolism" was obtained. Thirty normal subjects have been examined both by the open and closed circuits. The results agree with these reported last year in this report. The results will shortly be embodied in a paper.

Effect of Ammonia Ions on Blood Sugar.—It has been shown that oxidation of solutions containing glucose and ammonia in concentrations similar to those present in blood gives rise to the formation of urea; the reaction is represented by the following equation:—



It is possible that the same reaction takes place in the body and that hyperglycaemia is a protective mechanism against the excessive formation of ammonia, which is thereby converted into urea. Hovarth has obtained results pointing to this possibility. During the year experiments were carried out using ammonium hydroxide, ammonium bicarbonate, ammonium chloride, ammonium acetate and urease, with the object of ascertaining whether NH_4 ions always produced hyperglycaemia and whether this hyperglycaemia is proportional to the concentration of NH_4 ions. The bearing of this problem on diabetes will also be studied. So far the experiments have shown that ammonium hydroxide among these preparations definitely produces hyperglycaemia.

CHEMICAL DEPARTMENT.

A. Chemical Work.

Waters.—During the year under report 111 samples of water were examined chemically; 38 of these were also examined for their mineral constituents as well. The sources from which they were obtained and the extremes of variation in total solids and chlorides (as chlorine) are shown in the table. The samples are grouped into three divisions according to the sources from which they are derived; viz. surface waters, borings, and wells, the first including rivers, lakes, tanks, reservoirs, jack wells and tap supplies:—

Samples		Total solids Parts per 100,000		Chlorine Parts per 100,000		Potability	
		Highest	Lowest	Highest	Lowest	Fit	Unfit
Surface waters	.. 54	43·2	8·8	6·5	<i>Nil.</i>	8	46
Borings	.. 26	278·0	42·0	108·0	6·8	5	21
Wells	.. 31	520·0	23·2	122·0	0·8	1	24

Of the 111 samples examined, 14 were found to conform to the standards laid down in English text books on hygiene.

Other materials.—Human Milk—One sample was chemically examined and found to be of average composition.

B. Bacteriological Work.

During the year forty-four samples of water were examined bacteriologically. Of these 36 were samples of drinking water from rivers or reservoirs and were examined with a view to determine their fitness for potable purposes. In addition 8 samples of swimming bath water were examined, at the request of the Committee of the Breach Candy Baths.

The examination consisted in determining (i) the number of viable bacteria per cubic centimetre of the sample aerobically on nutrient agar, and (ii) the least quantity of the sample that was capable of producing acid and gas in MacConkey's neutral red bile salt lactose broth on two days' incubation at 37° C.

Drinking waters.—The sources from which the samples were derived are shown below :—

Surface water	..	..	35 samples.
Soda water	..	..	1 sample.

The bacteriological findings are shown in the following table :—

Total No. of aerobic bacteria per cc. of sample	Least quantity of sample fermenting MacConkey's broth							Total No. of samples
	No. fermentation in 100 cc.	100 cc.	10 cc.	1.0 cc.	0.1 cc.	0.01 cc.	0.001 cc.	
1-10	3	4	..	..	..	..	..	7
11-100	..	4	2	3	..	..	..	9
101-1000	..	1	6	2	1	..	1	11
1001-10000	..	..	1	2	2	..	1	6
Above 10000	..	..	1	..	..	1	..	2
Total No. of samples	3	9	10	7	3	1	2	35

From this table it will be seen that on the whole the total bacterial content and the *B. coli* content run parallel, i.e. when the former is low the latter is also low.

Three samples out of the six chlorinated waters examined conform to the English standard of bacterial purity.

About half the number of the samples of water may be considered as bacteriologically fit under Indian conditions.

Aerated water.—The sample did not contain any aerobic bacteria in 1 cc., nor did 100 cc. of the sample show the presence of any lactose fermenters.

Swimming Bath Water.—The 8 samples of water from the Breach Candy Swimming Bath were drawn from the outside bath, the inside bath, the settling tank, and the filter, at approximately six-monthly intervals.

It was considered desirable to avoid selective sampling and the samples were therefore taken without regard to the period which had elapsed since the water had been changed or treated. The following table shows the general results of the examinations :—

Source of sample	Total number of aerobic bacteria per cc. of sample drawn on		Minimum quantity of sample showing acid and gas in MacConkey's broth in sample drawn on	
	8th June 1929	17th December 1929	8th June 1929	17th December 1929
Outside Bath ..	44	4,000 (Deep left end). 16,000 (Deep right end).	0·1 cc.	0·01 cc. (Deep left end). 0·01 (Deep right end).
Inside Bath ..	10	5,000 (Shallow end). 400 (Deep end).	100 cc.	0·1 cc. (Shallow end). 10·0 cc. (Deep end).
Settling Tank ..	15		100 cc.	
Filter ..	60		10·0 cc.	

All the samples drawn on the first occasion in June, except one, met the standard of purity required for swimming bath water. Of the four samples drawn in December one sample only, that from the deep end of the inside bath, can be said partially to approach this standard of purity, the other three samples showing a high bacterial and coli content.

The first batch of samples was probably drawn after treating the water or cleaning the baths, while the second batch was probably drawn after the baths had been in use for some time.

ANTI-RABIC DEPARTMENT.

• The present report covers the period from 1st January to 31st December 1929. The outstanding feature of this year was the introduction of the Paris strain fixed virus in the preparation of anti-rabic vaccine and its use for treatment in this Institute only ; the out-centres were supplied with anti-rabic vaccine prepared from the Bombay, Kasauli and Paris strains.

From Table I which gives the statistics of persons treated at the Haffkine Institute, a comparison may be made of the mortality rate with the Paris strain in use during 1929 and that with the Bombay and Kasauli strains used in the previous years. It is evident that there is no appreciable difference in the mortality rates with these different strains.

The total number of persons who received the full course of anti-rabic treatment in this Institute during the year 1929 was 685, a decrease of 33 as compared with the number treated during the year 1928 ; there were, in addition, 151 persons who for various reasons did not complete the full course of treatment, and 565 persons were given advice but no treatment. This decrease and discontinuation of treatment was coincident with the prolonged strike of the mill working classes and to some extent may be attributed to, this. Two persons arrived with symptoms of hydrophobia and are therefore not shown on the register. Two persons died within 15 days of completion of treatment and three died after 15 days after completion of treatment, the latter being classed as failures giving a mortality rate of 0.44 per cent.

Out centres.—During the year 4,762 antirabic treatments were issued to the 43 out centres ; a statement of cases treated at the different centres during the year 1929, is published in Table “ A ”.

Health returns.—The apathy of persons treated towards the submission of the health returns is the most distressing part of the whole investigation. I attribute the above apathy and the resultant difficulty in obtaining health returns primarily to the illiteracy of the people, and secondarily to the large number of persons coming for treatment on their own initiative instead of through a responsible authority as is the case at Kasauli and Coonoor where patients are sent for treatment through authorities who can later on trace them. Out of 685 persons treated 649 belong to the labouring class, and it is from this class that the returns were not received. In spite of every effort made to find out the health of the persons treated, the following table will give one an idea of our difficulties :—

Health return for the year 1929.

Month	Number of cases treated	Number of health cards received before reminders were sent	Number of reminders sent	Number of replies received	Number of persons who did not reply	Returned by the Dead Letter Office
January	51	12	39	20	10	9
February	31	4	27	17	8	2
March	43	6	37	25	7	6
April	53	15	38	18	10	6
May	53	19	34	38	10	16
June	72	8	64	43	17	10
July	83	13	70	41	15	7
August	70	7	63	39	7	6
September	61	9	52	25	14	5
October	59	15	44	20	12	5
November	49	12	37	40	10	6
December	60	4	56			
Total	685	124	561	343	134	84

The above table shows that 18·2 per cent sent in their health returns without reminders. This number increases to 68·2 per cent after sending in reminders. 19·5 per cent. took no notice of the reminders, and 12·2 per cent. reminders were returned from the Dead Letter Office as the persons could not be traced.

Table A shows the number of cases treated at different out-centres and the health returns received from the Railway and Military and Native State centres and from those in charge of civil surgeons. The large number of returns (71·97 per cent.) from the three former compared with 31·88 of the latter substantiates what I had mentioned before about the necessity of a dispatching officer. The centres under the Civil Surgeons experience the same difficulties as we do in Bombay.

Veterinary.—193 antirabic treatments were issued to the various veterinary surgeons for treating dogs and other animals. The brains of 72 dogs and 5 cats were received from Bombay city and the adjoining suburbs. Two were found unfit for examination: in 20, there was no evidence of rabies; and in 55 brains, Negri bodies were present.

Brains of 106 dogs were received from various out stations. 12 brains were unfit for examination; in 21, there was no evidence of rabies and 73 brains were found to contain Negri bodies.

VACCINE DEPARTMENT.

Plague—

7,076 brews of 1000 cc. each were decanted.

260,287 bottles (approximate) were filled with vaccine, labelled, numbered and sealed.

16,622 bottles were tested for sterility.

1,248,560 doses of the prophylactic were in all sent to the Dispatch Department for packing.

Antirabic vaccine—

398 brews were decanted.

48,352 bottles were filled:—

	21 bottles of	100 ccs.
8,209	„	25 „
9,601	„	10 „
30,521	„	5 „

48,352

Labels were printed for these bottles. They were labelled, numbered and sealed.

930 bottles were tested for sterility.

91,188 doses or 6,513 treatments were sent to the Dispatch Department.

Other vaccines prepared—

3,550 ccs. Mixed T.A.B. Vaccine.

17,970 ccs. Cholera Vaccine.

Killed Bacterial Emulsions prepared—

400	ccs.	<i>B. typhosus</i> .
300	„	<i>B. paratyphosus</i> A.
700	„	„ B.
700	„	„ C.
500	„	<i>Micrococcus melitensis</i> .
400	„	<i>B. proteus</i> X 19.

Stock Cultures—

294 various stock cultures were subcultured at least once a month.

13 special cultures of *Pneumococcus* were subcultured on special media every 4 days. (Blood medium was prepared from time to time for the above cultures.)

Miscellaneous Work—

Decanting	3	flasks	of	saline.
	6	„	„	Glycerine Bile Peptone.
	2	„	„	Hydnocarpus oil.
	3	„	„	Citrate saline.
	4	„	„	Plague vaccine for immunity test.
	3	„	„	Distilled water.
	4	„	„	Bile.
	23	„	„	Horse serum.
	5	„	„	Carbolized saline.
	1	„	„	Quinine solution.
	2	„	„	Chicken-cholera Vaccine.
	30	„	„	Anti-cholera Vaccine.
	5	„	„	T.A.B. Vaccine.

Decanting 1 flask of Dead Emulsion of *B. typhosus*.

1	„	„	„	<i>B. paratyphosus</i> A.
2	„	„	„	„ B.
1	„	„	„	„ C.
1	„	„	„	<i>Micrococcus melitensis</i> .
1	„	„	„	<i>B. proteus</i> .
1	„	„	„	Plague B. 456.
1	„	„	„	„ 462.

Testing for sterility 133 bottles of various vaccines, etc.

Inoculation.—467 persons were inoculated against plague,

Teaching.—Eight medical men were taught the technique of plague inoculation.

Miscellaneous articles supplied :—

			No. of indents	Quantity supplied
Vaccines :—				
Mixed T.A.B.	..	..	148	2,635 cc.
Cholera	..	..	78	8793 cc.
Chicken Cholera	..	..	7	300 cc.
Dead Emulsions :—				
<i>B. typhosus</i>	..	..	41	329 cc.
<i>B. paratyphosus</i> A	..	..	33	256 cc.
„ B	..	..	29	216 cc.
„ C	..	..	6	17 cc.
<i>Micrococcus melitensis</i>	..	..	3	20 cc.
Culture Media :—				
Blood agar tubes	..	..	3	24 tubes.

				Number supplied
Living Cultures :—				
<i>B. anthracis</i>	..	..	..	1
<i>V. cholerae</i>	..	..	..	3
<i>B. coli</i>	..	..	..	1
<i>B. diphtheriae</i>	..	..	..	1
<i>B. dysenteriae</i> Flexner	..	..	..	2
<i>B. dysenteriae</i> Shiga	..	..	..	3
<i>Micrococcus melitensis</i>	..	..	..	1
<i>B. pestis</i>	..	..	..	5
<i>B. prodigiosus</i>	..	..	..	1
<i>B. proteus</i>	..	..	..	1
<i>B. paratyphosus</i> A	..	..	..	8
„ B	..	..	..	7
„ C	..	..	..	1
<i>B. typhosus</i>	..	..	..	8

Snake Venom.—Five indents for Cobra and Daboia venom were complied with.

Snakes.—69 Cobras, 10 Russell's Vipers, and 2 *Echis carinata* were received during the year. On an average 27 Cobras and 10 Russell's Vipers were taken out fortnightly for extraction of venom and feeding.

DIAGNOSTIC DEPARTMENT.

(1) By the General Diagnostic Department :—

Material examined	Positive	Negative	Total
A. <i>Blood</i> —			
(a) Films—microscopical examination,			
for malaria	4	45	49
,, microfilaria	..	1	1
,, differential leucocyte count	..	..	34
,, piroplasma (dog's blood)	..	2	2
(b) Cultures—for plague	6	3	9
,, other organisms (Typhosus etc.)	..	..	9
(c) Serological Examinations :—			
Wassermann reaction (including 11 of Cere-			
brospinal (Fluid)	630	919	1,549
Kahn Test	237	461	698
Blood grouping	..	..	17
Agglutination Test,			
for T. A. B.	29	53	82
,, Undulant fever	..	3	3
,, <i>B. coli</i> infection	..	2	2
Weil-Felix Reaction	1	4	5
(d) Total Cell Counts :—			
Red cells	..	..	3
Leucocytes	..	..	3
B. <i>Sputum</i> —			
For <i>B. tuberculosis</i>	6	20	26
,, Other organisms	..	..	26
C. <i>Urine</i> —			
General analysis	..	..	93
Cultures	..	..	46
D. <i>Faeces</i> —			
(a) Microscopical examination,			
for ova and cysts	..	27	27
,, T. B.	..	1	1
(b) Cultural examination,			
for miscellaneous organisms	..	..	14
,, cholera, typhoid and dysentery	5	25	30
(c) For occult blood	..	1	1
E. <i>Cerebrospinal Fluid</i> —			
For meningococcus	..	2	2
,, T. B. and other organisms	..	6	6
F. <i>Miscellaneous Smear Examinations</i> —			
Swabs for <i>B. diphtheriae</i>	5	25	30
Pus for <i>Gonococcus</i>	..	9	9
Pus for <i>B. tuberculosis</i>	..	3	3
Spleen pulp for Leishman-donovan bodies	1	..	1
Nasal discharge for Leprosy	..	1	1
Various material for <i>B. pestis</i>	48	62	110
G. <i>Miscellaneous Cultural Examinations</i> —			
Swabs for <i>B. diphtheriae</i>	7	12	19
Pus (various)	..	..	12
Blister fluid	..	..	2
Various pathological body fluids	..	..	7
Basundi (milk preparation)	..	..	1

Material examined	Positive	Negative	Total
H. <i>Miscellaneous Work</i> —			
Hair and scrapings for fungus (ringworm etc.)	..	4	4
Material for actinomycosis	..	1	1
Rideal-Walker Test	..	..	2
Cultures identified	..	..	3
Snakes identified	..	..	22
Autogenous vaccines prepared	..	..	121

The Wassermann Test employed is Method No. IV of the Medical Research Committee (M. R. C. Special Report Series, No. 14).

(2) By the Bio-chemical Department :—

	No. of cases
(a) Chemical Analyses of Blood—	
Non-protein nitrogen ..	25
Urea nitrogen ..	24
Uric acid ..	10
Creatinine ..	14
Fatty acids ..	6
Cholesterol ..	21
Phosphorus ..	8
Calcium ..	11
Sugar ..	19
(b) Chemical Analyses of Urine—	
General qualitative analyses ..	27
Quantitative analyses—	
Total nitrogen, urea, uric, acid, albumen, acetone bodies ..	28
Urea concentration test ..	2
Ambar's coefficient test ..	1
Free and conjugated phenol ..	1
(c) Basal Metabolic Rate Determination ..	4
(d) Miscellaneous Chemical Analyses—	
Faeces—fat estimation ..	2
Hæmoglobin determinations ..	12
Cerebrospinal fluids ..	3
Van den Bergh reaction ..	6
Urinary calculi ..	2
Ghee and cooking oil ..	3

PATHOLOGICAL SECTION.

Table showing the total number of Pathological Tissues received and examined during the year 1929

	No.
Non-malignant tumours ..	46
Malignant tumours ..	94
Tissues found to be normal ..	6
Miscellaneous pathological conditions ..	305
Received in a state unfit for examination ..	7
Total ..	458

Table showing the classification of Malignant Tumours and their Situation

		Carcinoma	Squamous Epithelioma	Sarcoma	Endothelioma	Basal Cell Carcinoma and Rodent ulcer	Total	Remarks
<i>Lymphatic System—</i>								
Cervical glands		1	..	..	..	..	1†	Secondary growth.
Axillary glands		1	..	..	..	..	1	Secondary growth.
<i>Respiratory System—</i>								
Maxillary antrum		1	..	..	..	..	1	
<i>Digestive System—</i>								
Gums		1	..	..	1	..	2	
Tonsils		1	..	..	..	..	1	
Salivary glands		..	..	..	4	..	4	"Mixed" tumours.
Peritoneum		1	..	..	..	..	1	Secondary growth to ovarian tumour.
Appendix		1	..	..	..	..	1	
Caecum		1	..	..	..	..	1	
Rectum		2	..	..	..	..	2	
Anus		1	..	..	..	..	1	
<i>Urinary Organs—</i>								
Kidney		1	..	..	..	..	1	From kidney pelvis.
Bladder		1	..	..	..	..	1	
<i>Male Reproductive Organs—</i>								
Prostate		1	..	..	..	..	1	
Testis		2*	..	1	..	..	3	*Teratomata.
Penis		4	1	..	..	..	5	
<i>Female Reproductive Organs—</i>								
Breast		11	..	..	..	..	11	
Ovary		2	..	..	1	..	3	
Uterus		3	..	..	..	..	3	One chorion epithelioma.
Cervix		3	5	..	..	..	8	
Vagina		1	..	..	..	..	1	
<i>Ductless Glands—</i>								
Thyroid		1	..	..	..	..	1	
<i>Special Senses—</i>								
Eye		..	..	2	..	..	2	One glioma.
<i>Skin and Subcutaneous Tissues—</i>								
Face		3	..	..	4	..	7	
Lips		2	..	2†	..	..	4	†Haemangioendothelioma.

		Carcinoma	Squamous Epithelioma	Sarcoma	Endothelioma	Basal Cell Carcinoma and Rodent ulcer	Total	Remarks
Head and Neck	..	..	1	..	..	2	3	
Forearm	..	..	1	..	..	..	1	
Foot	..	1*	2	..	..	..	3	*Melanotic Carcinoma.
Abdominal Wall	..	1†	..	..	..	1	2	†Secondary growth.
<i>Connective Tissues and</i>								
<i>Muscles—</i>								
Head and Neck	..	..	..	4	..	..	4	
Thorax	..	..	..	1	..	..	1	
Upper Extremity	..	..	..	2	..	..	2	
Lower Extremity	..	..	..	1	..	..	1	
Abdominal Wall	..	..	..	1	..	..	1	
Pelvis	..	..	..	1	..	..	1	
Lumbar Region	..	..	..	1	..	..	1	
<i>Bones and Joints—</i>								
Ribs	..	..	..	1	..	..	1	Chondro-sarcoma.
Forearm	..	..	..	1	..	..	1	
Jaws	..	..	..	2	..	..	2	One myeloma.
Knee	..	..	..	1	..	..	1	
<i>Unclassified—</i>								
Face	..	..	1	..	..	..	1	No details available.
Pelvis	..	..	1	..	..	..	1	Secondary growth.
Total	..	34	25	20	8	7	94	

ANTI-PLAGUE INQUIRY.

I. OBSERVATIONS ON THE CULTURAL CHARACTERS OF *B. Pestis*.

This study has been continued with special reference to the purity control of plague cultures used in the early stages of vaccine preparation.

It has long been the practice at this Institute to keep up the virulence of the stock strains of the plague bacillus used for vaccine preparation by passage through susceptible rats. In the first instance the bacillus is obtained by haemoculture from a human case of septicaemic plague. The broth culture of human blood is injected subcutaneously into a rat, death usually following in about two or three days. Passage of the strain of *B. pestis* is then effected by rubbing the spleen of this rat into the shaved skin of another rat. By this process the strain can be made to maintain its virulence indefinitely.

In order to prepare the seed for sowing the broth flasks used for the preparation of Haffkine's prophylactic, a sample of the heart blood of a passage rat dead of plague is drawn in a sterile capillary pipette and mixed with about one cubic centimetre of citrated saline. From this a series of tenth dilutions is made and plated on superimposed blood agar slopes, with a view to obtaining discrete colonies. These, when composed of *B. pestis*, become visible to the naked eye only after two days' incubation at 37° C., no colonies being visible after an overnight incubation. (It is interesting to note that the colonies of many other members of the Pasteurella group become visible to the naked eye after overnight incubation and some of the species are haemolytic. These characteristics along with the protein reactions greatly assist in the cultural identification of different members of this group of bacilli.) During the year under report 463 samples of the heart blood of passage rats were thus examined and in all samples it was found that the plague bacillus either grew in pure culture or that it predominated even though contaminants were detected. In every case the bacillus produced nitrite in peptone water culture and it invariably failed to produce hydrogen sulphide or indole, or haemolysis on blood agar.

In passing, it may be observed that the reactions, either protein or carbohydrate, as seen in liquid media, are indications of the presence of *B. pestis* but do not necessarily indicate the absence of a contaminant. In such cases the only way in which the presence of a contaminant can be detected with certainty is by the appearance of the culture on solid media.

The cultural control of the purity of the plague cultures used in the manufacture of Haffkine's prophylactic initiated by Col. Mackie in 1924, and subsequently developed so as to facilitate the detection of all possible contaminants, either in the heart blood of the "passage" rat, or in the seed, or in the resulting brew of vaccine, has been found to be of great assistance in meeting sudden and urgent demands for the vaccine.

This control has enabled the Institute to supply large quantities of antiplague vaccine of guaranteed purity and sterility in six weeks, while previous to the introduction and development of the cultural control at least two months or more notice was required before the Institute was able to comply with such requisitions.

Some contaminating organisms were met with which were liable to escape detection since they were found to be capable of growing side by side with the plague bacilli. Furthermore they grew as slowly as or even more slowly than *B. pestis* in the broth and certain of their cultural appearances grossly resembled those of the plague growth. In some cases, too, the growth being very transparent would readily escape detection in the surface growth of *B. pestis* on solid media.

The nitrite production by bacteria resulting from the oxidation of protein matter besides being a useful cultural test for the identification of *B. pestis*, has been found to differentiate the "smooth" variant of *B. avisepticus* from its "rough" variant.

Dr. Gore submitted a paper entitled "Protein Reactions of the Bacteria" to the Medical and Veterinary Section of the Indian Science Congress, 1930, and it will appear in an early issue of the Indian Medical Gazette.

II. THE STANDARDIZATION OF ANTI-PLAGUE VACCINE.

(a) *Preparation of a constant test virus for the determination of the immunizing value of the Plague Prophylactic.*—The routine method that has been used in this laboratory for measuring the relative immunizing value of one brew of plague prophylactic against another has made use of 0.003 mg. of the spleen of a plague rat as the test virus. It was shown in last year's report (p. 25) that this test virus contained a very variable number of organisms. Seven test viruses actually used in routine work were tested and the number of organisms per test dose was found to vary between 120 and 46,000. In a series of duplicate tests carried out on 11 samples of vaccine, using as the test dose 0.003 mg. of plague spleen, a variation in the immunity value of as much as 300 per cent. was observed. In Major Sokhey's and Dr. La Frenais' work, reported last year, a standardized test virus containing from 490 to 1,000 organisms was tried, but the results of a series of duplicate determinations were not entirely satisfactory. The work has been continued during the year under review and a more suitable test virus for the purpose has been developed.

The method used was as follows:—Blood agar slopes are planted with diluted heart blood of a rat dying on the third day after infection by the cutaneous route with a strain of *B. pestis* of known virulence. The strains, originally isolated from human cases, are kept up by regular passage from rat to rat. A slope showing perfectly discrete colonies after three days' incubation is selected and incubated at room temperature for a further period of four days. On the seventh day one entire colony is lifted, planted in 10 cc. broth and incubated for exactly 48 hours; half a cc. of this broth culture is then added to a fresh 10 cc. tube of broth. After incubating the second tube for 48 hours, serial tenth dilutions are made, and 1 cc. of the dilution 1 in 100,000 is used as the dose of test virus for each rat. Ten test viruses so made gave colony counts between 40 and 100 organisms per cc.

For the starting point a strain of *B. pestis* is selected of such virulence that 7-10 organisms per cc. of the diluted broth culture prepared in the way described above will kill 70 per cent. to 100 per cent. of susceptible rats within ten days. This test gives a measure of the virulence of the strain and for the test virus ten times this minimal number of organisms is used.

Five immunity determinations on the same sample of vaccine, using this standard test virus, have now been carried out and immunity percentages of 23, 25, 25, 26 and 32 respectively, were evaluated. The same sample of vaccine when tested with a test virus 100 times as strong gave immunity percentages of 9, 14 and 27, a difference of more than 200 per cent. between the lowest and the highest figure. These

experiments, so far as they go, indicate that excessive and variable amounts of the test virus should be avoided, otherwise dependable and comparable results cannot be obtained. It is proposed to multiply the experiments during the course of the present year (1930).

In all the above experiments susceptible Madras rats were used as the test animal, and all injections are given subcutaneously.

(b) *The accuracy of the method of counting organisms.*—The method described above depends on the technique used for making counts of the organisms in the broth cultures used. It is found that colony counts made by planting the dilutions on blood agar (rabbit blood) give fairly accurate results.

Of the 48 hour broth culture, the following serial dilutions are made in nutrient broth, using serum pipettes :—(A) 1 in 100, (B) 1 in 1,000 (C) 1 in 10,000, (D) 1 in 100,000 and (E) 1 in 1,000,000. Dilutions (D) and (E) are planted on blood agar slopes, using four slopes for each dilution and 1-20 cc. of each dilution for each slope. At the same time dilution (B) is planted on plain agar slopes, as a control (*vide* Gore's test referred to in last year's report); if any growth appears on these slopes on the second day, contamination is suspected and further tests are made. The colonies on blood agar are counted after three days' incubation. A very large number of these counts has now been done; it is found that they are consistent among themselves and that (E) dilution invariably gives within narrow limits 1-10 the count obtained from (D) dilution.

These colony counts have been checked against direct counts under the microscope using the Petroff-Hausser Bacteria Counter. The counts of three different cultures gave the following results by the two methods :—

					Colony counts millions per cc.	Direct counts millions per cc.
1st culture	..	..	..	..	52	56
2nd culture	..	..	..	..	78	85
3rd culture	..	..	..	..	79	95

The difference between the colony counts and the direct counts is explained by the fact that in spite of vigorous shaking, a number of short chains persist; under the microscope each member of a chain is counted separately, but when counted as colonies, obviously each chain gives rise to a single colony only. Moreover, under the microscope the viable and non-viable organisms are not differentiated.

(c) *Ratio of growth on agar to that on blood agar.*—The year before last, when an attempt was first made to develop a constant test virus on a similar plan, the method failed because dependable colony counts could not be obtained on the plain agar slopes then employed. Dr. Gore's observation that blood agar is a better medium for the growth of

B. pestis has rendered this work possible. Since then Major Sokhey and Dr. La Frenais have determined the ratio of growth on the two media. They have found that (B) dilution (1 in 1,000) of a 48 hour broth culture gives no growth on a plain agar slope, while on blood agar fairly accurate colony counts are obtainable with dilutions (D) (1 in 100,000) and (E) (1 in 1,000,000). Their undiluted 48 hour broth cultures are usually found to contain between 40 and 100 million organisms per cc., so that (B) dilution theoretically contains between 40,000 and 100,000 organisms per cc. When 1-20 of a cc. of this dilution, i.e., from 2,000 to 5,000 organisms, is planted on each slope, no growth is obtained, so that *B. pestis* in this concentration fails to grow on plain agar slopes of the size used. When, however, 1-20 cc. of dilution (A) (1 in 100) i.e., 20,000 to 50,000 organisms is planted out, varying amounts of growth occur on the plain agar slopes.

(d) *The effect of the addition of blood and blood products to nutrient broth on the growth of B. pestis.*—(i) The broth cultures obtained by the technique described above under the preparation of the test virus give between 40 and 100 millions per cc. after 48 hours' incubation. But if to the broth is added $\frac{1}{2}$ cc. of rabbit blood per 10 cc. of broth, the growth is about three times as profuse. It has been found, however, that *B. pestis* grown in broth enriched by the addition of blood rapidly loses its virulence and if repeatedly subcultured in such a broth its virulence for susceptible rats is entirely lost. In one experiment 40 million organisms of a fortieth subculture in blood broth, each subculture incubated for 48 hours, failed to kill rats, while 50 organisms of the original broth culture killed 100 per cent. of the rats experimented with.

(ii) Experiments were also carried out to determine the effect of the addition to nutrient broth of inactivated (heated) rabbit's blood. The growth obtained was as profuse as that obtained by the addition of untreated blood, and the organisms retained their virulence.

(iii) The effect on the growth of *B. pestis*, of a preparation of haemoglobin, kindly supplied by the Digestive Ferment Company of Detroit, was then tried. Agar slopes, enriched with this preparation of haemoglobin were prepared for the purpose of colony counts and it was found that these slopes gave exactly the same number of colonies as blood agar slopes. It is not known if this preparation of haemoglobin contains serum protein also.

It would seem that the two last experiments, if confirmed, indicate a ready method for the preparation of media suitable for the growth of *B. pestis* and one that is free from many of the disadvantages attending the use of fresh blood for this purpose.

(e) *Measuring the relative virulence of the different strains of B. pestis.*—The method of counting organisms in a broth culture permits the determination of the minimal number of organisms of a particular strain required to kill 80-100 per cent. of susceptible rats (i.e., the M.L.D. of the particular strain). The minimal lethal doses are inversely proportional to the virulence of the strain and using this method great differences in the virulence of different strains have been met with,

III. PARASITOLOGY OF PLAGUE.

(a) *The rôle of the non-pectinate rat-fleas in India.*—The flea factor in plague epidemiology has been studied throughout the year on the lines indicated in last year's report.

The technique adopted for continuous transmission experiments has proved successful in maintaining a specifically pure flea population and in preventing invasion by *Tyroglyphid* mites. The epizootic has continued throughout the year with both *X. cheopis* and *X. astia* respectively and the sequence of events appears to have been similar in the two cases. It must be emphasised that this experiment is purely qualitative and ignores several factors, such as the longevity of starved infected fleas and the specific flea index. It appears, however, to be a practicable means of determining the extreme limits of temperature and humidity favourable for transmission by different species of fleas. Climatic conditions in Bombay have, therefore, allowed uninterrupted transmission by both *X. cheopis* and *X. astia*. It is proposed to test *X. brasiliensis* by the same method.

A large number of plague infected fleas from the epizootic pits has been examined, with a view to obtaining information regarding the occurrence of Bacot's blocking phenomenon in the tropics. The proportion of blocked fleas has been extremely small. The material has, however, provided some interesting information. Thus, blocked *astia* have been obtained at a mean temperature of over 80° F. and some of these have proved capable of infecting a susceptible animal with a single bite. It may be noted that complete blocking is not essential for infection to occur, as a partially blocked flea may obtain a blood meal and infect the host at a single bite.

Comparative transmission experiments on carefully controlled lines have been continued. The essential point is, that the fleas are infected on the same septicaemic animal, and are afterwards identified and tested in groups according to species and sex. *X. brasiliensis* has proved a particularly regular transmitter of the infection under these conditions. This series of experiments will be recorded in detail elsewhere.

Dr. Gore's work, in connection with the mixed flea experiments, indicates that a large proportion of infected fleas of both sexes of the three species of *Xenopsylla* harbour the plague bacillus for at least a fortnight, even during the off-season for plague, and that there is no marked change in the virulence of the bacillus in any of the fleas during that period.

Infection of fleas on a dead rat had previously been considered as highly improbable. One experiment has, however, demonstrated the possibility of such an event. The plague infected rat had been dead for about half an hour. Forty fleas were allowed access to it, and twelve succeeded in feeding. Two of these were proved by culture to contain plague bacilli twenty-four hours later.

The bionomics of the various fleas has been studied on comparative lines. The method adopted for securing laboratory-bred fleas, in pure species, has continued to give highly satisfactory results. It may be

mentioned that individual fleas of the three species have been kept alive for many weeks on an exclusive diet of human blood. The longest periods noted were:—

X. cheopis 162 days, *X. astia* 53 days, *X. brasiliensis* 127 days.

A rat-flea survey, on a small scale, of Bombay city has been carried out over the period of a year. Monthly samples have been obtained from six selected areas. Detailed information regarding the specific flea index, with seasonal and regional variations will shortly be available. All the samples have yielded *cheopis* and *astia*, but *brasiliensis* has been a rare catch, except in one area. There is some evidence that both *R. norvegicus* and *Gunomys* species are more attractive hosts for *astia* than *R. rattus*.

An inquiry was received from Hyderabad State, regarding the value of keeping dogs in plague-infected houses with the idea of detaching the infected fleas from human beings. A short series of experiments to demonstrate the susceptibility of dogs to plague, and the attractiveness of dogs to rat-fleas, was therefore carried out. It was concluded that although dogs are highly immune to plague, they are not particularly attractive to rat-fleas. The mere presence of a dog in a plague-infected house, along with human beings and the usual additional fauna, would probably have no appreciable influence on the occurrence of human plague. The Plague Commission came to a similar conclusion regarding the presence of cats in plague infected houses. The rat-fleas may occasionally feed on cats and dogs, and be transported by them from house to house, but they probably do not tend to remain for long on the foreign host.

(b) *Observations on a cultural method for detecting B. pestis in fleas fed on plague rats.*—Since the discovery that blood agar is an ideal medium for the growth of *B. pestis* and that it is easily available in the form of the superimposed blood agar slopes, Dr. Gore has been able to follow up the bacteriological examination of individual fleas used by Major Webster in plague transmission experiments. For this work, he used 0.5 per cent. bile salt nutrient agar as the basis for making blood agar, the advantage being that the presence of bile lessens the susceptibility of blood agar to aerial contamination.

A large number of fleas have been cultured for the determination of the presence of *B. pestis* in the proventriculus and stomach contents. The technique is simple—it does not take more than a minute to put up one flea, a period less than that required for staining and examining a film of the contents—and gives positive results more often and with greater certainty than can be obtained by means of the staining method. In several fleas, bacteria other than *B. pestis* were found to be present either alone or growing side by side with plague bacilli. The number of plague bacilli per flea varied approximately from 1,000 to 10,000,000. This subject is being further studied and will form part of the Plague Transmission Inquiry.

Having thus found the culture method of detecting plague bacilli in infected fleas superior to the microscopic method, an experiment was

instituted in collaboration with Major Webster and Dr. Chitre to determine the value of the culture method as compared with the animal test—the second procedure in vogue in such work.

Forty fleas from one of the plague transmission experiments were dissected and a saline suspension of the proventriculus and stomach contents used for culture and injection into rats; the quantity used for culture was approximately one-fifth of that used to inject rats subcutaneously. The results obtained are shown in the following table:—

Species of Flea	No. of fleas	Method of detecting plague	Number of fleas showing plague on the day								Number of fleas showing plague	
			2nd	3rd	4th	5th	6th	7th	21st		+	—
									+	—		
<i>X. cheopis</i>	10	Culture	2								2	8
Female.		Rat			1				1		2	8
<i>X. cheopis</i>	3	Culture	3								3	0
Male.		Rat		2		1					3	0
<i>X. astia</i>	10	Culture	9								9	1
Female.		Rat	1	6	1			1			9	1
<i>X. Astia</i>	8	Culture	5								5	3
Male.		Rat		1			1	1	1	1	4	4
<i>X. brasiliensis</i>	6	Culture	6								6	0
Female.		Rat		3	2				1		6	0
<i>X. brasiliensis</i>	3	Culture	3								3	0
Male.		Rat			1		1			1	2	1
Total	40	Culture	28								28	12
		Rat	1	12	5	1	2	2	3	2	26	14

From this table it will be seen (1) that while plague bacilli were cultivated from 28 fleas, *post-mortem* signs of plague were found in 26 rats also (2) that plague bacilli were detected culturally within two days in all the 28 fleas, while only one rat died within this period; 22 rats died between the 3rd and 7th days after injection; 5 rats which survived injection of material giving positive culture tests were killed after three weeks; all showed abscesses, from which in three cases plague bacilli were obtained.

This method of culture is therefore superior in every way to both the procedures so far used to determine the presence of plague infection in fleas.

IV. ANTI-PLAGUE SERUM.

The possibility of preparing a potent anti-plague serum in India has been the subject of study by Dr. Naidu and his assistants. This work has been continued throughout the year.

The successful therapeutic results obtained in rabbits experimentally infected with plague by the use of an immune serum prepared from rabbits — animals susceptible to experimental infection with plague—encouraged further study. In order to obtain a therapeutic serum on a sufficiently large scale to be of practical value it is obvious that the immunization of large animals is necessary. From the results obtained in rabbits it seemed probable that the animal of choice for preparing a potent anti-serum would be one which is naturally susceptible to infection with organisms of same type as that against which immunity is desired and in which some evidence of the process of immunity can be demonstrated. Haemorrhagic septicaemia occurs in nature in, and is a fatal disease among sheep and cattle and is caused by members of the *Pasteurella* group of organisms which are very closely allied to *B. pestis*. The immunization, therefore, of sheep and calves for the preparation of anti-plague serum was commenced in 1928 and was continued throughout the present year. In June 1929, one of the calves which had received 38 weekly injections of plague culture, and two of the sheep which had received 60 and 41 weekly injections, were bled and with the sera obtained comparative tests were carried out along with the Pasteur Institute anti-plague serum, which is now in general use.

Agglutination Tests.—The normal sera of sheep, calves and horses do not agglutinate the plague bacillus. The immunized sheep's sera agglutinated living cultures of plague in a dilution of 1 in 64 (supernatant fluid water clear), the calf serum agglutinated the bacilli in a dilution of 1 in 256 (supernatant fluid water clear) while the anti-plague serum of the Pasteur Institute failed to agglutinate the bacilli even in a dilution of 1 in 2. Thus, the Pasteur Institute serum does not agglutinate our local strains of *B. pestis*.

Precipitin Tests.—Experiments carried out with the filtrate of a broth culture of *B. pestis* of six weeks' growth, showed the presence of precipitins in the sera of immunized sheep and calf, but failed to demonstrate their presence in the Pasteur Institute serum. Thus, the Pasteur Institute serum does not contain any precipitins for our local strains.

Therapeutic Tests.—The therapeutic value of the sera of immunized sheep was estimated along with the Pasteur Institute serum, in rats infected subcutaneously with plague. Out of 100 rats in which both the immunized sheep serum and the test virus were administered simultaneously and subcutaneously, 13 rats died within 7 days of infection with a case mortality of 13 per cent., among 100 rats similarly infected and treated with the Pasteur Institute serum there was a mortality of 52 per cent., and among the 100 controls there was a mortality of 83·3 per cent. When the test animals were kept under observation for a period of one month from the time of infection,

this difference between the mortality obtained with these sera was mostly lost, as many rats died during this interval.

Out of 300 infected rats in which the treatment was commenced after an interval of 24 hours after infection, with immunized sheep serum in doses of 1, 2 or 3 cc. subcutaneously, the mortality at the end of 7 days of infection was 33 per cent., whereas, among the 300 rats similarly infected and treated with the Pasteur Institute serum in the same doses, there was a mortality of 77·3 per cent.; the mortality among the untreated controls was 93 per cent. Here also, when the test animals were kept under observation for a period of one month, this difference in the mortality between the two groups was considerably reduced.

The therapeutic value of the immunized calf serum was tested along with the Pasteur Institute serum, in rabbits which were infected subcutaneously with plague, but in which the serum was administered intravenously. Out of the 100 rabbits infected, in which the treatment with calf serum was commenced at the end of 48 hours and 72 hours, respectively, after infection, there was a mortality of 21 per cent. at the end of 15 days after infection; among the 100 rabbits similarly infected and treated with the Pasteur Institute serum there was a mortality of 58 per cent. In these animals no further mortality occurred although they were kept under observation for a period of one month. The mortality among the untreated rabbits was 91 per cent. within 15 days of infection.

From these results it would appear that the potency of different anti-plague sera is best estimated in plague-infected rabbits by the intravenous administration of sera.

Anti-plague sera of the Lister Institute.—At the suggestion of Dr. Petrie, comparative experiments on lines planned out by him are being carried out in rats with two anti-plague sera sent out by him, along with the Pasteur Institute serum, normal horse serum and our locally immunized sheep and calf sera. The results so far seem to suggest that both sheep and calf sera have greater anti-toxic and curative value than the two sera of the Lister Institute.

Protective value of sera.—This was estimated by the subcutaneous injection of serum in doses of 1, 2, 3 or 4 cc. and after an interval of 48 hours the test animals were infected with the test dose. The results of two comparative experiments showed that at the end of seven days after infection, the protection among rats treated with sheep serum was nearly 99 per cent., with calf serum it was 90 per cent., with Lister Institute "ROWLAND" serum, 70 per cent. and with the Pasteur Institute serum, 30 per cent.; the Lister Institute "ARMISTICE" serum and normal horse serum conferred little or no protection.

Minimal Lethal Dose of Plague Toxin.—The minimal lethal dose for rats of the toxic filtrate of a broth culture of plague grown for six weeks was estimated in two sets of experiments. The results showed that with 2·5 cc. of the filtrate there was a mortality of 95 per cent., in rats within 7 days of injection by the subcutaneous route.

Anti-Toxic Value of Sera.—This was estimated by injecting subcutaneously in rats a mixture consisting of the minimal lethal dose of the toxin with serum in increasing doses 1·5 cc., 2 cc., and 2·5 cc. The results of two comparative experiments showed that within seven days of injection of this mixture, there was no mortality among the rats treated with sheep and calf sera; there was a mortality of 30 per cent., among those treated with the Lister Institute "ROWLAND" serum, 43 per cent. among those treated with the Lister Institute "ARMISTICE" serum, and nearly 52 per cent. among those treated with the Pasteur Institute serum. Normal horse serum possessed little or no anti-toxic property.

Curative Value of Sera.—To estimate the curative value of the sera, rats were injected subcutaneously and simultaneously with the test dose of the virus, and serum in increasing doses of 1 cc., 2 cc. and 4 cc., respectively. The results of two comparative experiments showed that at the end of 7 days after infection there was a mortality of 3·3 per cent., in rats which received sheep serum; a mortality of 5 per cent. among those which received calf serum, a mortality of 31 per cent., in those treated with the Lister Institute "ROWLAND" serum, and a mortality of 65 per cent. among those treated with the Pasteur Institute serum. With the Lister Institute "ARMISTICE" serum and normal horse serum there was a mortality of 93 per cent. The mortality among the controls during the same period was 90 per cent.

As previously noted, in these experiments also the mortality among the test animals considerably increased when the period of observation was extended to a month, and the differences in the mortality rates with the different sera were lost.

Immunization of sheep, bulls and buffaloes are now in progress, and when sufficient serum becomes available, it is proposed to carry out further therapeutic tests in human cases of plague.

V. CHEMOTHERAPY OF PLAGUE.

The drugs under investigation in this part of the inquiry are the halogenomercuriphenols. Twelve of these compounds are theoretically possible and all have been synthesized. Nine of them were hitherto unknown. The results of purely chemical interest have been embodied in a paper published in the Journal of the Indian Chemical Society.

These twelve compounds have since been prepared in amounts sufficient to meet requirements in the subsequent work of evaluating their toxicity and their action on plague infected animals.

The bactericidal value of these twelve compounds was tested against *B. pestis in vitro* and some correlation between their chemical structure and chemical composition on the one hand, and their bactericidal activity on the other, were noted. One of the factors which determines the bactericidal activity of any compound *in vitro* is the number of organisms with which a definite amount of the compound is brought in contact; an attempt was made to prepare by cultural methods standard

suspensions of *B. pestis* containing approximately equal numbers of organisms as estimated by colony counts. This attempt was not successful and it has therefore not been possible, up to the present, to determine the absolute bactericidal value of the individual compounds.

The relative bactericidal value of the different compounds has, however, been estimated by testing the whole series of drugs against one and the same emulsion, standardized by opacity. The tests were all carried out at the same time and under the same experimental conditions.

In general it may be said that the chloro derivatives are more potent than the bromo, iodo and fluoro derivatives, and that the para compound in any one of the four series is more potent than either the ortho or di compound.

From preliminary experiments in which the onset and subsequent progress of the infection following an injection of *B. pestis* in animals was studied, it appeared that the pure bred English rabbit was the animal of choice for this work. Difficulties in breeding these animals have caused a certain amount of delay, but these have now been overcome.

Work is now in progress to determine (i) the toxicity of the drugs to rabbits, and (ii) the therapeutic action of the drugs on plague infected rabbits.

INQUIRY ON MATERNAL MORBIDITY AND MORTALITY IN CHILDBIRTH.

A. ANÆMIA OF PREGNANCY.

I. *Dietetic Survey of certain classes of women in Bombay City.*—This investigation was limited to women of the child bearing age. The method of survey necessitated visiting women on five consecutive days. Exact notes were made of diet eaten, housing, wages, etc., and a blood count taken at the time of visiting. The following groups of women were examined :—

- (1) 20 healthy women of the upper middle class.
- (2) 155 women of the hospital class :
 - (a) 70 healthy women recently delivered of healthy living children.
 - (b) 25 women recently delivered of premature infants but otherwise apparently healthy.
 - (c) 20 Hindu mill-workers.
 - (d) 40 women who had been treated in Hospital for “pernicious anæmia of pregnancy”.

The following generalizations can be made from the results obtained :—

- (1) The diet of the upper middle class Hindu women is both adequate and well balanced and contains a good supply of the essential food elements, such as animal protein, vitamins and salts. This class is not anæmic.

(2) All the Hospital women examined are, relatively to the better class women studied, both quantitatively and qualitatively ill fed and anæmic.

(3) The diet is deficient in (i) calories, (ii) total and animal protein and fat, (iii) all fresh fruit and vegetables and therefore in (iv) vitamins A, C, and D, and (v) in salts. The supply of vitamin B varied with the nature of the grains eaten.

(4) The Hindu mill-workers have the most marked deficiency in fats and animal protein but eat good grains.

(5) The diet of the mothers of premature infants is markedly deficient in vitamin B.

(6) It is suggested that among a population generally deficient in vitamins A and C an important factor determining the incidence of "pernicious anæmia of pregnancy" over and above such exciting cases as pregnancy may be the proportion of fat and vitamin B in the diet.

This line of investigation is being continued, specially with reference to the mill-workers and mothers of premature infants.

II. *Animal Experiments.*—This work was started in the Nutritional Research Laboratories, Coonoor, and is being continued at the Haffkine Institute.

Rats were used for the earlier work. Blood standards were established for the Coonoor stock. This laborious piece of work was essential as there is a great range in 'normal' blood findings from stock to stock.

Feeding experiments led to the production of a severe anæmia, specially in the female animals, and this anæmia was further shown to be a true *Bartonella* anæmia occurring in intact animals. The diets used were both synthetic and natural, the latter being based on those in common use among our anæmic patients in Bombay. The only common factor in the diets was apparently a relative deficiency in vitamins A and C, but we recognise that there may be some other associated deficiency.

A series of experiments with splenectomized rats showed that all the common stock was infected with *Bartonella Muris ratti* and therefore liable to develop the disease. Further experiments showed that the exhibition of liver extract, fresh spleen or vitamin A concentrate failed to protect the rats from this anæmia after splenectomy. All attempts to culture the organisms from the blood failed.

Although we have been able to produce a severe megalocytic anæmia in rats, especially in female animals, we were not satisfied that this had a direct bearing on the question of the etiology of "pernicious anæmia of pregnancy" as the experimental anæmia was an infective one though produced by dietetic means, and so far we failed to obtain any evidence of a primary infection in our human cases. We are therefore repeating our experiments using monkeys instead of rats, as these animals are not liable to develop a *Bartonella* anæmia, except after inoculation of

infected blood. The work has been in progress since Christmas and in one old monkey a severe anæmia has been produced which is responding to liver extract. This work is to be continued.

III. *Chemical work.*—Various new lines of treatment are being tried but it is too early to report on these. Further attempts have been made to isolate a pathogenic organism from the blood and excreta of cases, but up to date these have failed.

B. OSTEO-MALACIA.

The serum calcium and inorganic phosphate of the blood have been estimated in a further series. Cases seen in Bombay with a few exceptions belong to the low phosphate class, the serum calcium being only slightly reduced. The response to treatment has been followed by repeated blood examinations. It is hoped to publish these results shortly in conjunction with Dr. Jhirad of the Cama Hospital.

C. INVESTIGATIONS INTO CAUSES OF PREMATURE BIRTH.

A clinical inquiry has been carried out during the year. One hundred women delivered of premature children were examined clinically; a careful history was taken and a Kahn test and blood count done in all cases. The results of this work are to be published shortly.

D. REPORT ON STILLBIRTH INQUIRY.

This investigation, which was commenced in February 1929, has now been completed, and a full report is in course of preparation. The latter will consist of two main divisions :—

(1) A summary of two hundred foetal and neonatal *post mortems*, checked by hospital statistics as to causes of death, from Bombay, Madras and Calcutta, whence the material itself was obtained.

This checking of results is essential, as of course *consecutive post mortems* were unobtainable, with the result that the series contains an unduly high proportion of premature, macerated fetuses, which is the type of case in which syphilis is frequently found.

(2) The classified results of an All-India stillbirth questionnaire for 1929 which was sent out—

(a) by Dr. A. C. Scott, C.M.O., W.M.S., to all the Dufferin hospitals, and

(b) by Dr. Thomson, to other large provincial and Mission hospitals throughout India.

Appendices will show full data *re* individual cases in certain groups of stillbirths, and also detailed figures relative to the questionnaire.

I. In the stillbirths series, a full *post mortem* examination was made in each case, when material for ordinary microscopical examination was taken from the seven most important organs, and for Levaditi's method, from lung, liver, spleen, kidney, and supra-renal. Slides were also made from the lower end of the femur, and when obtainable, from

the placenta. The examination of these slides has now been completed and in the first 200 Levaditis, 13·5 per cent. were positive for *T. pallidum*. A full clinical history of the mother was obtained in all cases, when possible, together with films of the peripheral blood for malarial examination, and blood-tests for syphilis. The results of examination of the urine were also noted together with the blood-pressure. In a number of cases, e.g., those sent from a distance and instances of maternal deaths, histories and tests were necessarily incomplete.

Special sections will be given in the report, as to eclampsia, syphilis, anæmia of pregnancy, malaria, etc., as causes of foetal death, and data *re* the probable incidence of these diseases in the three Presidency cities have been obtained as far as possible.

In connection with the inquiry as to syphilis, a series of one thousand parallel Kahn's and Wassermann Tests of material taken by the staffs of the various maternity hospitals in Madras, were carried out at the King Institute, Guindy, by kind permission of the Director. The results of 500 of these already tabulated show an incidence of 8 per cent. of syphilis, judged on the blood tests alone. In Bombay, a series of 250 Khans has just been completed by Dr. H. Patil, at the Cama Hospital, and shows an incidence of 9·2 per cent.

At the Eden Hospital, Calcutta, a series of 500 Wassermanns has been commenced, and it is hoped to include part of this series in the report.

Although all the work on the 200 *post mortems* has been finished, the results have not yet been fully summarized. The figures from the hospital statistics of Bombay, Madras and Calcutta, although necessarily only approximate (in the absence of *post mortems*), show very interesting comparisons with the English statistics.

Thus labour complications *in toto*, account for some 30 per cent. of all the stillbirths and neonatal deaths, as compared with 35 per cent. in England; ante-partum hæmorrhage shows a lower incidence in India, while in the latter country, "maternal states" including tropical diseases, show a figure ranging from six to nine times as much as the English figure (which is 2·5 per cent.). "Foetal states", such as monstrosities, are much less frequent in India, while prematurity *per se*, varies in the great cities, from 12 to 19 per cent., as compared with 3·6 per cent. in England.

In the absence of *post mortems* and blood-tests the hospital figures for proved syphilis are of course hopelessly inadequate.

Toxæmias of pregnancy show figures in the three Presidency cities, varying from 6 to 17 per cent., the frequent occurrence of severe eclampsia in Calcutta being noteworthy.

In addition to the *post mortem* cases, a series of some one hundred placenta of stillborn infants have been examined in Bombay and Madras, clinical data being obtained in all these instances. Fifty cases are also being specially investigated for malaria in the placenta, films being taken from the maternal peripheral blood, the cord, and the placenta.

Twenty-five cases were taken during the non-malarial season, and twenty-five during the malarial-season. The examinations are being carried out by Major J. A. Sinton, V.C., I.M.S., Director, Malaria Survey of India, Kasauli, and by Dr. Maneck Mehta at the Haffkine Institute, Bombay. The series is of course much too small for statistical work, but owing to lack of time, more could not be attempted. So far, the results show a very small percentage of infected placentae, compared with the heavy incidence in other malarial countries, e.g., in West Africa.

II. In the classification of the All-India questionnaire three sets of tables are being worked out for each main cause of stillbirth or neonatal death, viz. :—

(a) Condition of the child at birth (i.e. macerated, fresh stillborn, or neonatal death),

(b) Parity of the mother,

(c) Religion,

while all the main causes are being shown as percentages, for comparison of one place with another. Valuable results are thus being obtained as to the geographical distribution of the main causes of death. It is hoped to show the latter in map-form, for the year 1929, although for accuracy, such causes should be classified over a period of years, to avoid errors due to the annual variation of certain types of disease, e.g., eclampsia. Owing to lack of time and personnel, it was, however, impossible to deal with more than one year's cases.

The *post-mortem* work has been carried out in Bombay and Madras, some thirty cases being also sent from Calcutta.

TUBERCULOSIS INQUIRY.

This inquiry was continued at this Institute throughout the year under review by Dr. Soparkar and his assistants.

Surgical Tuberculosis in India.—Study of the type of bacilli causing surgical forms of tuberculosis in India was continued. Material from 111 cases comprising glandular and bone and joint tuberculosis was collected from different hospitals. In several instances the material proved to be nontuberculous and 56 strains have so far been isolated. Fifty-one out of these have been studied as to their character, and with the exception of two cases mentioned previously, in one of which avian tubercle bacillus was isolated from the cervical glands of a young girl, and in the other a bovine type of bacillus, the rest of the strains were found to be of the human type. One of these, however, derived from a female breast was atypical and possessed the characters of an attenuated eugonic human strain. The remaining strains are under investigation. It will be fair to conclude from the results so far obtained that the bovine tubercle bacillus plays a negligible part in the causation of surgical forms of tuberculosis in this part of India. This would appear to be in conformity with the general finding of infrequency of tuberculosis among cattle in the western presidency.

Material from other parts of India, however, needs to be similarly investigated, especially from the Punjab where recent findings (see below) have shown that the chances of infection with the bovine bacillus are proportionately much greater.

Incidence of tuberculosis among cattle in India.—Tuberculosis among cattle is generally held to be rare in this country. The highest percentage of infection hitherto recorded has been about 3 to 3·5 at Ferozepur (Taylor). The prevalence in India of surgical forms of tuberculosis in about the same proportions to the pulmonary disease as occurs in England, where the former type of disease is found to be caused in a large majority of cases by the bovine bacillus, suggested this investigation and an attempt was made to determine the incidence of the disease among cattle.

Material was collected from the slaughter houses at Ferozepur and Lahore. As it was not possible under the conditions of work in the slaughter houses to make a thorough examination of each carcass, examination was restricted to the thoracic glands alone. These were sectioned and examined with the naked eye for lesions of tuberculosis. Smears were also examined for presence of tubercle bacilli and where no organisms were detected the suspicious material was further subjected to animal inoculation.

The animals examined consisted of cows, bullocks, buffaloes, and young calves. Excluding young calves the total number of animals examined was 1,115 out of which 255 or 22·85 per cent. were found definitely infected with tuberculosis as revealed by the presence of gross lesions in the glands and tubercle bacilli in smears made from them. The percentage of infection varied somewhat in different kinds of animals examined. Thus out of 357 cows 68 or 19 per cent. were found infected; out of 117 bullocks 37 or 31·6 per cent. were infected; and out of 642 buffaloes as many as 150 or 23·4 per cent. were found infected. These figures approximate those obtained in European countries where tuberculosis in cattle is known to be prevalent, and even exceed those in some parts of Europe and America and *pari passu* would increase the danger of infection of human beings with bacilli of bovine origin. The control and eradication of the disease among cattle in this part of India, therefore, calls for serious consideration.

Filtrability of the Tubercle bacillus.—Experiments on the question of the existence of filter passing forms of tubercle bacilli were continued and altogether 32 experiments were done with material from different sources, e.g., cultures of different types and of varying ages, tissues of animals dying of experimental infection and pathological material from tuberculous cases. Except in one instance noted previously, in no case did the animals inoculated with the filtrate develop lesions of tuberculosis. In several cases lesions closely simulating those of genuine tuberculosis were observed in the spleen of guinea pigs, but on further investigation they were found to be due to infection with a paratyphoid-like organism. In many cases hypertrophy of the

lymph glands in the neighbourhood of the seat of inoculation was noticed, but no definite evidence of tuberculosis was detected and passage experiments have been done and are in progress to see if this will ultimately develop into tuberculosis.

Experiments with B. C. G.—Experiments with B. C. G. (*Bacillus Calmette Guérin*) to test its (1) non-pathogenicity, (2) immunizing property were continued during the year.

Experiments on Guinea pigs.—The results of experiments carried out on guinea pigs indicated that although lesions resembling those produced by virulent tubercle bacilli are produced in some animals by inoculation of living B. C. G. culture, the lesions are not reinoculable and the strain in this sense is avirulent.

Experiments on Rabbits.—The general result of experiments showed that B. C. G. failed to produce progressive tuberculosis in these animals, but in two instances evidence of such infection was noticed. These two rabbits, previously inoculated with B. C. G., died, one 276 days and the other 449 days after intravenous inoculation with 20 mgms. of culture on glycerin-potato. Both animals showed definite tuberculous lesions in the organs and numerous acid fast bacilli in smears and a strain was isolated from one of them and on study was found to be of the bovine type. (It may be noted here that B. C. G. was originally derived from a bovine strain.) The results appeared to indicate that in exceptional cases of particularly susceptible animals B. C. G. may possibly prove to be virulent and produce fatal tuberculosis.

It should, however, in fairness, also be mentioned that although the animals were all along kept in separate cages and away from other animals infected with virulent bacilli there was an exception to this of a short interval of three days during a railway journey when all animals were kept in two or three crates. This circumstance may afford a possible explanation of the tuberculous infection found since this may have been contracted through close association with other infected animals. The explanation, however, is not wholly satisfactory, as if this supposition were correct, it will show a poor immunizing power of B. C. G. in that a three day contact with other infected animals, three and a half months after protective inoculation, led to the development of acute tuberculosis in the vaccinated animals. It may be noted that although a number of observers support Calmette's claim as to the innocuousness of B. C. G. a small group of investigators have observed progressive tuberculous lesions to follow inoculation of B. C. G. and Noble holds that under exceptional conditions B. C. G. is capable of producing fatal tuberculosis in laboratory animals.

Immunizing property of B. C. G.—Experiments were made on rabbits in which the animals were vaccinated by both subcutaneous and intravenous routes and two months later were given test doses of 1/10th, 1/100th, 1/1,000th, and 1/10,000th of mgm. of virulent bacilli. Results showed that when tested on rabbits B. C. G. affords only a slight protection resulting only in some prolongation of the life

of the vaccinated animals, for even those inoculated with the smallest test dose died of severe tuberculosis; further, that vaccination by the intravenous route appeared to confer a somewhat greater immunity than by the subcutaneous method.

It was shown in the last year's report that in the course of immunity experiments on cattle most of the calves vaccinated with B. C. G. died of acute generalized tuberculosis after a test dose of 5 mgms. of bovine bacilli given intravenously.

In the experiments carried out by the Ukraine Commission the same test dose of another strain of bovine bacilli (Vallée strain) failed to produce any serious disease in the vaccinated animals. In the more recent experiments of Obukhovski similar results were obtained in the vaccinated animals but when seven unprotected control animals were inoculated with the same Vallée strain they did not die. They were killed 2 to 7 months after inoculation and showed evidences of only chronic tuberculosis.

The absence in the control animals of the acute disease that usually follows intravenous inoculation of 5 mgms. of a virulent bovine strain is explained by the author on the assumption of the presence in the inoculated animals of a latent natural infection which the tuberculin test carried out at the commencement had failed to reveal. The result may, however, also be explained by the low virulence of the culture employed for test inoculation. This is supported by the experiments carried out by Rankin under the National Research Council of Canada who obtained similar results after intravenous inoculation of 5 mgms. of a one month old culture of tubercle bacilli (Vallée Strain) into unvaccinated animals and the author observes that the "results indicated that the strain of bacillus tuberculosis Vallée used, according to the condition of the experiment, was not capable of causing generalized tuberculosis in even unprotected animals" and that "the experimental results might have been much clearer had the infecting agent used had greater invasive powers".

In order to test this point a culture of the Vallée strain used by the Ukraine Commission has recently been obtained from Professor Tzekhnovitzer.

Virulence tests on rabbits have been done with this strain and the experiments are in progress; the results so far obtained show that the Vallée strain from the Ukraine is of decidedly lower virulence than the strain employed by us in our experiments on cattle.

The following paper was read before the Indian Science Congress, 1930: "A Note on the Innocuity and the Immunizing Property of B. C. G. (Bacillus Calmette Guérin)."

LIST OF PAPERS PUBLISHED DURING THE YEAR.

1. An investigation of the Value of the Complement Reaction in Sprue utilizing *Monilia psilosis* (Ashfordi) as Antigen: by Fairley, N. H., and Jasudasan, F. *Ind. Jour. Med. Res.* XVI, 4, p. 861.

2. Chemistry and the Science of Healing ; by Caius, J. F. *Grant Med. Coll. Mag.*, April 1929.
3. Lo que son y lo que no son las Serpientes ; by Caius, J. F. *Iberica*, XXXI, p. 777, Mayo 1929.
4. The Toxicity of the Venoms of Indian Scorpions, I. *Butheolus melanurus* Kessler. *Jour. Bom. Nat. Hist. Soc.*, XXXIII, 4, October 1929.
5. Donde moran las Serpientes ; by Caius, J. F., *Iberica*, XXXII, p. 803, Novembre 1929.
6. Earth-eating and Salt-licking in India ; by Caius, J. F. and Barucha K. H. *Jour. Bom. Nat. Hist. Soc.*, XXXIII, 3, May 1929.
7. Halogenomercuriphenols ; by Caius, J. F. and Wadia, J. H. *Jour. Ind. Chem. Soc.*, VI, 4, August 1929.
8. A note on the Anophelines found in Baroda Camp ; by Webster, W. J., *Ind. Med. Gaz.*, LXIV, p. 197.
9. The Anatomy of the Indian *Xenopsylla* Larvae ; by Webster, W. J., *Ind. Jour. Med. Res.*, XVII, 1, p. 90.
10. Observations on Rat-fleas and the Transmission of Plague, Part I ; by Webster, W. J. and Chitre, G. D., *Ind. Jour. Med. Res.*, XVII, 3, p. 699. (Sent for publication June 1929.)
11. The Relative Toxicity and Immunizing Value of Haffkine's Plague Prophylactic and other Anti-plague Vaccines compared ; by Naidu, B. P. B. and Shamsher Jung. *Ind. Jour. Med. Res.*, XVII, 1, p. 199.
12. Preparation of a Potent Anti-plague Serum in India ; by Naidu, B. P. B., Shamsher Jung and Kamakaka, K. H., *Ind. Jour. Med. Res.*, XVII, 4, p. 1259. (Sent for publication November 1929.)
13. Studies in "Pernicious Anaemia" of Pregnancy. Part I. Preliminary Report ; by Wills, Lucy, and Mehta, Maneck M. *Ind. Jour. Med. Res.*, XVII, 3, p. 777. (Sent for publication June 1929.)
14. A Case of Bovine Tubercle Bacillus Infection in Man in India ; by Soparkar, M. B., *Ind. Jour. Med. Res.*, XVII, 2, p. 574.
15. The Superimposed Blood Agar Slope ; by Gore, S. N., *Ind. Med. Gaz.*, August 1929.

The following papers were read at the last session of the Indian Science Congress, held at Allahabad in January 1930 :—

1. Protein Reactions of the Bacteria ; by Gore, S. N.
2. A note on the Innocuity and the Immunizing Property of B. C. G. (*Bacillus Calmette Guérin*) ; by Soparkar, M. B.

APPENDIX

Statistics of Anti-rabic Department.

TABLE I.

*Statistics for persons treated at Haffkine Institute, Parcel,
Bombay, 1922-1929.*

Year		Total treated	Failures		Deaths		Indians			Europeans			Cases extracted advice cases and incomplete treatment
			Total No.	Per cent.	Total No.	Per cent.	Total No. treated	Deaths	Per cent.	Total No. treated	Deaths	Per cent.	
1922	..	429	4	0·93	4	0·93	385	4	1·09	64	..	..	212
1923	..	845	9	1·06	10	1·18	730	10	1·37	115	..	..	523
1924	..	587	7	1·19	10	1·70	525	9	1·71	62	1	1·61	513
1925	..	625	5	0·8	6	0·96	583	6	1·02	42	..	..	355
1926	..	648	4	0·6	5	0·76	588	5	0·87	60	..	..	611
1927	..	729	5	0·68	6	0·82	678	6	0·88	51	..	..	644
1928	..	718	6	0·83	6	0·83	679	6	0·88	39	..	..	614
1929	..	685	3	0·43	5	0·73	650	5	0·77	35	..	..	716

TABLE II—Europeans.

*Classification according to number and depth of wounds, intervention
of clothing and cauterization.*

Deep	Bare	Uncau- terized	0	1	2	3	4	5	6	7	8	9	Total	Dea- ths	Morta- lity	
+	+	+	..	3	1	..	..	..	..	..	1	..	5	..	..	
+	+	—	..	2	1	1	1	..	..	1	1	..	7	..	..	
+	—	+	..	..	..	..	..	..	..	..	..	..	..	..	..	
+	—	—	..	..	1	1	..	..	..	..	..	1	3	..	..	
—	+	+	10	2	..	..	..	..	..	..	..	..	12	..	..	
—	+	—	..	1	1	1	1	..	..	..	..	..	4	..	..	
—	—	+	..	..	..	..	..	..	..	..	..	..	..	..	..	
—	—	—	..	..	..	1	1	..	1	1	..	..	4	..	..	
Total ..			..	10	8	4	4	3	..	1	2	2	1	35	..	..

TABLE III—*Europeans.**Classified according to position and depth of wounds.*

Deep	Bare	Leg	Trunk	Arm	Multiple	Face	Multiple face	Total	Deaths	Mortality
+	+	1	..	11	..	..	..	12	..	..
+	—	3	..	..	..	..	..	3	..	..
—	+	2	..	14	..	..	..	16	..	..
—	—	4	..	..	..	..	..	4	..	..
Total ..		10	..	25	..	..	..	35	..	..

TABLE IV—*Europeans.**Classified according to number of wounds and lateness of arrival.*

Days late		0	1	2	3	4	5	6	7	8	9	Total	Deaths	Mortality
0—3 days	..	4	4	2	4	2	..	1	1	2	1	21	..	..
4—7 days	..	3	2	1	..	1	..	..	1	..	..	8	..	..
8—14 days	..	3	1	..	..	..	..	..	..	..	..	4	..	..
15—21 days	..	..	1	1	..	..	..	..	..	..	..	2	..	..
Total		10	8	4	4	3	..	1	2	2	1	35	..	..

TABLE V—Indians.
Classified according to number and depth of wounds, intervention of clothing and cauterization.

Deep	Bare	Uncau- terized	0	1	2	3	4	5	6	7	8	9	10	11	12	14	17	20	Total	Deaths	Mortality
+	+	+	..	30	11 (1)	9 (1)	7	4	2	1	2	..	..	..	..	1	..	..	67	..	..
+	+	—	..	53	51	37	26	20	15	7	7	1	3	2	..	..	1	1	224	2	0.80%
+	—	+	..	6	3	2	..	1	..	1	..	..	..	..	..	..	..	..	13	..	..
+	—	—	..	7	7	11	11	3	2	2	..	..	..	..	..	..	1	..	44	..	..
—	+	+	36	10	7	4	1	..	..	(1)	..	..	..	..	..	..	..	..	58	..	..
—	+	—	2	38	48	22	15	7	3	5	1	..	1	1	..	..	..	..	138	1	0.72%
—	—	+	1	2	..	1	1	1	..	..	..	..	..	..	..	..	..	..	6	..	..
—	—	—	..	19	32	16	14	5	9	1	1	1	1	..	1	..	..	..	100	..	..
Total ..			39	160	159	102	75	41	31	17	11	2	5	3	1	1	2	1	650	3	0.48%

TABLE VI—Indians.

Classified according to number and position of wounds.

Position	0	1	2	3	4	5	6	7	8	9	10	11	12	14	17	20	Total.	Deaths.	Mortality.
Leg	3	109	(1) 118	71	50	27	22	(1) 10	8	1	4	2	1	1	..	..	427	2	0.47
Trunk	1	2	8	6	2	2	1	..	..	..	..	..	..	..	..	..	22	..	..
Arm	32	46	26	18	18	9	5	5	1	..	..	1	..	..	1	1	163	..	..
Multiple	2	1	3	3	1	2	2	2	..	..	..	..	..	..	1	..	17	..	..
Face	1	2	3	(1) 4	4	1	1	..	..	1	..	..	..	..	..	..	17	1	5.88
Multiple face	..	..	1	..	..	..	..	..	2	..	1	..	..	..	..	..	4	..	..
Total	39	160	159	102	75	41	31	17	11	2	5	3	1	1	2	1	650	3	0.46

TABLE VII—Indians.

Classified according to number of wounds and lateness of arrival.

Days late	0	1	2	3	4	5	6	7	8	9	10	11	12	14	17	20	Total.	Deaths.	Mortality.
0-3 days	21	88	(1) 124	(1) 79	61	33	28	(1) 13	9	1	5	3	1	..	2	1	469	3	0.64
4-7 days	12	44	15	11	5	4	2	4	1	1	..	..	..	1	..	..	100	..	..
8-14 days	3	13	7	8	4	3	1	..	1	..	..	..	..	..	..	..	40	..	..
15-21 days	2	6	9	3	1	..	..	..	..	..	..	..	..	..	..	..	21	..	..
22-30 days	..	3	2	1	..	..	..	..	..	..	..	..	..	..	..	..	6	..	..
31-39 days	..	1	..	..	1	..	..	..	..	..	..	..	..	..	..	..	2	..	..
40-50 days	..	3	2	..	1	..	..	..	..	..	..	..	..	..	..	..	6	..	..
51-60 days	1	..	..	..	2	..	..	..	..	..	..	..	..	..	..	..	3	..	..
70-80 days	..	1	..	..	..	1	..	..	..	..	..	..	..	..	..	..	2	..	..
90 days	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..
Total	39	160	159	102	75	41	31	17	11	2	5	3	1	1	2	1	650	3	0.46

TABLE VIII—*Indians.**Classified according to position and depth of wounds.*

Deep	Bare	Leg	Trunk	Arm	Multiple	Face	Multiple face	Total	Deaths	Mortality
										Per cent.
+	+	(1) 191	4	73	7	(1) 14	2	291	2	0.69
+	-	38	7	9	3	..	..	57	..	..
-	+	(1) 112	3	72	6	3	..	196	1	0.51
-	-	86	8	9	1	..	2	106	..	..
Total		427	22	163	17	17	4	650	3	0.46

TABLE IX—*Indians.**Classified according to position of wounds and lateness of arrival.*

Days late	Leg	Trunk	Arm	Multiple	Face	Multiple face	Total	Deaths	Mortality
									Per cent.
0-3 days ..	312(2)	17	109	11	16(1)	4	469	3	0.64
4-7 days ..	54	1	38	6	1	..	100	..	..
8-14 days ..	30	1	9	..	..	..	40	..	..
15-21 days ..	15	2	4	..	..	..	21	..	..
22-30 days ..	6	..	..	..	..	..	6	..	..
31-39 days ..	2	..	..	..	..	..	2	..	..
40-50 days ..	3	1	2	..	..	..	6	..	..
51-60 days ..	3	..	..	..	..	..	3	..	..
70-90 days ..	1	..	1	..	..	..	2	..	..
90 days ..	1	..	..	..	..	..	1	..	..
Total ..	427	22	163	17	17	4	650	3	0.46

TABLE X—All Classes.

Classification of patients by probability of rabies in the biting animals.

Class	Sub-class I Bitten on head or face	Sub-class II Bitten through bare skin anywhere except on the head or face	Sub-class III Bitten through cloth- ing	Sub-class IV Licks on any part of the body	Total	Deaths	Morta- lity per cent.
Class "A" bitten or licked by animals proved rabid..	3	39	8	31	81	..	..
Class "B" bitten or licked by animals certified rabid.	1	3	2	1	7	..	..
Class "C" bitten or licked by animals suspected rabid ..	17(1)	417(2)	136	27	597	3	0.50
Total ..	21	459	146	59	685	3	0.43
<i>Europeans</i>							
Class "A" bitten or licked by animals proved rabid ..	..	7	2	6	15	..	..
Class "B" bitten or licked by animals certified rabid.	..	1	..	1	2	..	..
Class "C" bitten or licked by animals suspected rabid ..	..	13	3	2	18	..	..
Total ..	..	21	5	9	35	..	..
<i>Indians</i>							
Class "A" bitten or licked by animals proved rabid ..	3	32	6	25	66	..	..
Class "B" bitten or licked by animals certified rabid.	1	2	2	..	5	..	..
Class "C" bitten or licked by animals suspected rabid ..	17(1)	404(2)	133	25	579	3	0.51
Total ..	21	438	141	50	650	3	0.46

TABLE XI.
Cases developing Hydrophobia within 15 days of completion of treatment.

Case No.	Age	Sex	Animal	Number and position of bite	Whether on bare skin or through clothing	Whether cauterized or not	Period between bite and cauterization	Date of commencement of treatment	Number of days late	Duration of illness	Interval between bite and death	Remarks
1	2	3	4	5	6	7	8	9	10	11	12	13
726	28	M	Dog	8, arm	Bare	Cauterized	9 hours	1st - July 1929.	0 day	1 day	25 days.	
1,156	11	M	Dog	4, face	Bare	Cauterized	24 hours	19th October 1929.	1 day	1 day	27 days.	

TABLE XII.

Cases developing *Elysiophobia* after 15 days of completion of treatment—"Failures."

Case No.	Age	Sex	Animal	Number and position of bite	Whether on bare skin or through clothing	Whether cauterized or not	Period between bite and cauterization	Date of commencement of treatment	Number of days of late	Duration of illness	Interval between bite and death	Remarks
1	2	3	4	5	6	7	8	9	10	11	12	13
76	7	M.	Dog	3, face	Bare	Cauterized	24 hours	19th January 1929.	1 day	1 day	103 days.	
354	10	M.	Dog	7, leg	Bare	Cauterized	26 hours	20th March 1929.	1 day	1 day	48 days.	
1,063	40	F.	Dog	2, leg	Bare	Cauterized	3 hours	28th September 1929.	0 day	1 day	49 days.	

TABLE XIII.

Showing the number of persons who came for treatment each month during the year 1929.

Month	Dis-continued treatment	Europeans		Indians	
		Treated cases	Advice cases	Treated cases	Advice cases
January ..	18	..	5	51	62
February ..	5	4	6	27	49
March ..	12	..	2	43	77
April ..	16	3	2	50	52
May ..	12	..	9	53	40
June ..	7	9	1	63	45
July ..	21	4	6	79	33
August ..	11	5	2	65	19
September ..	10	1	7	60	36
October ..	16	1	1	58	37
November ..	13	3	..	46	41
December ..	10	5	2	55	31
Total ..	151	35	43	650	522=1,401

TABLE XIV.

Showing the animals which bit, scratched or licked the patients.

Species of animal		Number of persons bitten, scratched or licked	Deaths
Dog ..	..	657(3)	3
Jackal ..	..	1	..
Cat ..	..	14	..
Monkey ..	..	4	..
Human beings ..	..	5	..
Pig ..	..	1	..
Horse ..	..	2	..
Zebra ..	..	1	..
Total ..		685	3

TABLE XV.

The following table shows the classes of population from which patients were derived :—

European Civilians—

In Government employ ..	2	
Others	33	
		35

Indian Civilians—

In Government employ ..	26	
Others	624	
		650
Total ..		685

Details of persons who died of Hydrophobia.

Case No. 1.—A boy aged 7 years was bitten on 18th January 1929 by a stray dog. This dog bit four other persons. Appeared for treatment 24 hours later; one deep lacerated wound in the forehead, one deep on the upper margin of orbit, and one superficial wound on face. Received the full course of treatment. Developed hydrophobia on 30th April 1929 and died on 1st May 1929.

Case No. 2.—A boy aged 10 years was bitten on 28th March 1929 by a dog rabid according to history. Appeared for treatment 24 hours later; seven superficial tooth marks on the right leg. Developed hydrophobia on 14th May 1929 and died on 15th May 1929.

Case No. 3.—A man aged 28 years was bitten on 1st July 1929 in the morning. Seven hours later he reported here for treatment. One deep wound on the middle finger and one deep on the ring finger and one deep on the palm, and two deep lacerated wounds on left thumb. The wounds were disinfected and cauterized. Underwent full course of treatment. Developed hydrophobia 11 days after completion of treatment and died the next day.

Case No. 4.—A woman aged 40 years arrived 3 hours after being bitten on 28th September 1929. The wounds were disinfected and cauterized. Two deep punctured wounds on the left ankle, one deep on the sole of the left foot. Underwent the full course of treatment; developed hydrophobia on 14th November 1929 and died on 16th November 1929.

Case No. 5.—A boy aged 11 years arrived on 19th October 1929 one day late, from Palghar. Four deep lacerated wounds on lip which was badly damaged and three deep communicating wounds on the left cheek. Underwent full course of treatment; developed hydrophobia within fifteen days of completion of treatment and died the next day, i.e., 15th November 1929.

TABLE A.

Statement of cases treated at different centres during 1929.

Centres	Total number of anti-rabic treatments received by the centre	Number of persons who received the full course of treatment	Number of persons not fully treated	Number of reports received from persons alive and well at least for 6 months from date of treatment	Died during treatment	Died less than 15 days after completion of treatment	Died more than 15 days after completion of treatment	Number of persons from whom no health report was received
<i>Centres under Civil Surgeons.</i>								
Ahmedabad ..	580	576	49	..	..	..	..	576
Ahmednagar ..	101	101	..	..	..	..	..	101
Akola ..	130	125	6	7	..	1	1	116
Baroda ..	113	83	29	42	1	..	..	40
Belgaum ..	120	155	7	152	..	1	..	2
Hoshangabad ..	205	196	9	191	..	1	4	..
Hyderabad ..	55	49	8	3	..	..	..	46
Indore ..	515	479	36	62	5	3	6	403
Jalgaon ..	87	83	4	54	..	..	..	29
Jubbulpore ..	433	406	27	16	1	1	2	386
Karachi ..	270	225	45	224	1	..	..	..
Karwar ..	2	2	..	2	..	..	..	..
Nagpur ..	368	341	27	251	..	..	2	88
Nasik ..	90	85	21	70	..	..	1	14
Poona ..	311	359	120	..	..	..	..	359
Raipur ..	123	110	13	25	1	..	3	81
Rajkot ..	429	420	9	107	..	..	5	308
Ratnagiri ..	4	4	..	4	..	..	..	..
Sholapur ..	44	41	15	1	..	..	..	40
Surat ..	55	40	37	26	..	..	..	14
Total ..	4,035	3,880	462	1,237 31.88 per cent.	9	7	24	2,603 67.09 per cent.
<i>Centres under Railways, Military, and Indian States.</i>								
Ahmednagar ..	2	2	..	2	..	..	..	..
Ajmer ..	175	146	2	143	..	..	..	3
Barwani ..	6	6	..	5	..	..	1	..
Bombay :								
Byculla ..	508	524	11	304	..	..	..	220
Colaba ..	9	9	..	..	..	..	..	9
Cambay ..	17	15	2	15	..	..	..	..
Chhota Udepur ..	2	2	..	2	..	..	..	..
Hubli ..	22	22	..	..	..	..	..	22

TABLE A—*contd.*

Centres	Total number of anti-rabic treatments received by the centre	Number of persons who received the full course of treatment	Number of persons not fully treated	Number of reports received from persons alive and well at least for 6 months from date of treatment	Died during treatment	Died less than 15 days after completion of treatment	Died more than 15 days after completion of treatment	Number of persons from whom no health report was received
<i>Centres under Railways, Military, and Indian States—contd.</i>								
Jhahsi	58	48	8	46	..	..	..	2
Jubbulpore	20	20	..	..	..	..	..	20
Kolhapur	27	21	6	21	..	..	..	..
Mhow	31	38	1	33	..	..	..	5
Nasirabad	6	6	..	6	..	..	..	..
Nova Goa	116	105	7	103	2	..	..	..
Poona	19	18	1	18	..	..	..	..
Quetta	65	45	7	41	..	..	..	4
Total	1,083	1,027	45	739 71.97 per cent.	2	..	1	285 27.75 per cent.

